Effect of Dietary Supplementation of Choline from Different Sources on Performance and Economics of Commercial Broiler Chicken

Arman S. Ghasura¹, Fulabhai P. Savaliya², Rais M. Rajpura³, Atul B. Patel³, Nikesh J. Bhagora⁴, Nishant M. Patel⁵

Abstract

An experiment was conducted to investigate the effect of dietary supplementation of choline from different sources on the performance and economics of commercial broiler chicken. Day old broiler chicks (Vencobb, n=216) were randomly distributed into nine experimental groups with four replicates of six birds each. The experimental groups (T₁ to T₉) were fed with diet containing choline chloride 60% @ 1000 g/ton, 750 g/ton, and 500 g/ton feed in T₁ (Control), T₂, and T₃ ration. Similarly, herbal choline @ 1000 g/ton, 750 g/ton and 500 g/ton feed was given in T₄, T₅, T₆ ration, and phosphatidylcholine @ 1000 g/ton 750 g/ton and 500 g/ton feed-in T₇, T₈, T₉ ration, respectively. The trial was carried out in deep litter pen for 42 days. During the whole experiment, the mortality pattern of the birds was within the permissible limits and revealed no adverse effects of feeding choline from different sources in diet. The study results indicated that the supplementation of choline chloride 60% @1000 g/ton feed (Control group) showed higher body weight and body weight gain compared to other rations. Total feed consumption and feed conversion ratio of birds fed with treatment rations supplemented with different choline sources were at par among the rations. The return over feed cost was highest in broilers fed a diet with choline chloride 60 % @ 1000 g/ton feed.

Keywords: Broiler, Choline chloride, Economics, Herbal Choline, Performance parameters.

Introduction

Choline, which mainly occurs as phospholipids and has been re-discovered as vitamin B₄, plays a crucial role in a number of biological functions for poultry. Choline is a solid and hygroscopic alkaline chemical, aminoethyl alcohol with three methyl groups called 2-hydroxyethyl trimethylammonium. Choline is an important factor in producing and maintaining cell membranes and organelles, such as mitochondria and microsomes, and in bone cartilage maturity. It is an essential neurotransmitter (Ferguson et al., 2004) that helps the transmission of nerve impulse and participates in the formation of acetylcholine. Choline is also very essential for fat metabolism and referred as lipotropic agent, promote the flow of fat and bile to and from the liver which reduces an undesired build-up of fat in the liver (fatty liver) and also promotes its transport as a lecithin, or enhances its use in the liver itself (Wen et al., 2014). Choline is an active donor of labile methyl group in the synthesis of betaine from homocysteine and in many methylation reactions. The nutrients choline, betaine, and methionine are often beneficial in enhancing liver function and detoxification reactions.

Choline is present in food as free choline or in complex forms like phosphocholine, glycerophosphocholine, sphingomyeline, or phosphatidylcholine. Phosphatidylcholine is responsible for removing lipids from the liver in the body as they are essential for the synthesis of very-low-density lipoproteins (VLDL), that carry fat to the tissues on the periphery. Herbal choline is a complement of herbal poultry food containing selected herbs that are rich in natural choline and in stable and highly bioavailable conjugated choline. They also have small amounts of methionine, betaine, and chromium traces. These small amounts of methionine and betaine provide methyl groups readily accessible for
the methylation reactions needed by the system. Herbal choline has a stronger physiological impact to optimise energy absorption and the use of nutrients to avoid energy transformation into lipids and to avoid fatty liver syndromes (Koujalagi et al., 2018). In view of the above fact, the present study was conducted to investigate the effect of dietary supplementation of choline from different sources on performance and economics of commercial broiler chicken.

**Materials and Methods**

The study was undertaken to investigate the effect of choline from different sources on the performance of commercial broiler chicken (Vencobb). The experiment was carried out on 216 straight run day-old commercial broiler chicks of a single hatch. The chicks were randomly distributed to nine treatments, consisting of 24 chicks in each treatment. Each treatment consisted of four replicates with six chicks per replicate. The brooding and rearing of chicks were carried out in a deep litter system using standard management and health care practices. The experiment was conducted for a period of six weeks. Nine treatment diets (T1 to T9) were prepared and offered ad libitum to each respective treatment group as starter and finisher mash. The nine treatments were: T1 (Control) diet with choline chloride 60% @1000 g/ton feed, T2 diet with choline chloride 60% @ 750 g/ton feed, T3 diet with choline chloride 60% @ 500 g/ton feed, T4 diet with herbal choline @ 1000 g/ton feed, T5 diet with herb choline @ 750 g/ton feed, T6 diet with herbal choline @ 500 g/ton feed, T7 diet with phosphatidylcholine @ 1000 g/ton feed, T8 diet with phosphatidylcholine @ 750 g/ton feed and T9 diet with phosphatidylcholine @ 500 g/ton feed.

Weekly body weight of the individual bird was recorded in the morning hours before feeding with the help of digital weighing balance at day old age (BW0), at 1st (BW1), 2nd (BW2), 3rd (BW3), 4th (BW4), 5th (BW5) and 6th (BW6) weeks of age. Weekly body weight gain was calculated by subtracting the live body weight of the previous week from that of the current week and recorded in grams. Data regarding feed intake was calculated on a weekly basis. FCR was calculated by dividing the feed intake by weight gain. Livability was calculated based on recorded mortality that occurred during different stages.

The economics of each treatment group was calculated replicate-wise after the end of the experiment as a return over feed cost (ROFC). ROFC was calculated as per formula considering the selling price of birds as Rs. 80/per kilogram live weight and the actual cost of different treatment diets. The data were analyzed using a completely randomized design as per Snedecor and Cochran (1994). Means of replicates under each treatment were considered for analysis.

**Results and Discussion**

The mean body weights (g) at different ages, i.e., BW0, BW1, BW2, BW3, BW4, BW5 and BW6 are presented in Table 1. At the end of 3rd, 4th, 5th & 6th week of age, dietary treatments T1 (Control, Choline chloride 60% @ 1000 g/ton) showed significantly (p < 0.05) higher body weight (i.e., 758.17 ± 20.9, 1299.38 ± 26.85, 1838.38 ± 31.50 and 2452.60 ± 44.40 g, respectively) compared to other dietary treatments. Whereas significantly (p < 0.05) lower body weight was observed in the group of the birds fed with T9 (phosphatidylcholine @ 500 g/ton) diet. The body weight was found to be gradually increased with increased level of choline from different sources, except herbal choline (T9), where lower level was beneficial. The present finding was in close agreement with the results of Fouladi et al. (2008), Kathirvelan et al. (2013), and Igwe et al. (2015).

The weekly body weight gains (g), body weight gain during the pre-starter phase (BWG0-3), starter phase (BWG3-4), finisher phase (BWG4-6) and overall experimental period (BWG0-6) are presented in Table 2. The BWG differed significantly in birds fed with different rations during 2nd, 3rd and 4th week of age, as well as during pre-starter, finisher phase and overall of 6 weeks period. At the end of the experiment, dietary treatments T1 (Control) showed significantly (p < 0.05) higher body weight gain (2406.14 ± 44 g) as compared to other dietary treatments. Whereas, lowest body weight gain was observed in the group of birds fed with T9 ration (2027.46 ± 53.74 g). Like BW, the BWG was also gradually increased with increasing choline levels from different sources, except herbal choline (T9), where lower level was beneficial. The overall results of present experiment indicate that choline chloride 60% @1000 g/ton feed supplementation in diet as is fed routinely improved body weight and body weight gain significantly at the end of 6th week of age, and there was no advantage of reducing the level or replacing with phosphatidyl or herbal choline. These findings were in agreement with the results observed by Jadhav et al. (2008), Kumar and Sharma (2014), Sharma and Ranjan et al. (2016), and Khose et al. (2018).

The data on weekly feed consumption (g/bird) recorded during 1st to 6th week of age, as well as for pre-starter (0-2 weeks), starter (3-4 weeks) and finisher (5-6 weeks) phase and overall experimental period (0-6 weeks) are presented in Table 3. The total feed consumption (g/bird) at all these ages, including from 0-6 weeks of age in birds fed with T1 to T9 diets were statistically similar. However, apparently, the highest feed consumption was observed in birds fed with T1 ration (4268.52 g) and the lowest in the birds fed with T9 ration (3900.91 g). Further, among herbal and phosphatidylcholine sources, the birds fed middle levels (@ 750 g/ton feed) consumed more feed than higher or lower levels. These findings were similar to the results reported by Chatterjee and Misra (2004), Sharma and Ranjan (2016) and Farina et al. (2017), who observed that supplementation of different choline sources had no influence on the average daily feed consumption.
Table 1: Mean weekly body weights (g) of broilers fed with different treatment diets

| Body weight gain | Treatments | T_1(Control) | T_2 | T_3 | T_4 | T_5 | T_6 | T_7 | T_8 | T_9 | SEM | CD at 5% | CV% |
|------------------|------------|--------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|------|------|
| BWG_{0-1}        | 125.79 ± 9.65 | 121.71 ± 5.72 | 116.58 ± 1.02 | 120.58 ± 3.00 | 117.34 ± 4.24 | 124.00 ± 4.75 | 116.69 ± 5.62 | 113.67 ± 3.73 | 108.29 ± 5.85 | 5.33 | NS | 9.02 |
| BWG_{1-2}        | 200.03 ± 7.94 | 166.71 ± 10.81 | 174.62 ± 14.50 | 194.58 ± 5.00 | 177.86 ± 13.53 | 205.99 ± 13.94 | 169.95 ± 16.06 | 160.68 ± 8.03 | 146.15 ± 10.63 | 11.37 | 33.00 | 12.82 |
| BWG_{3-4}        | 385.89 ± 3.17 | 319.00 ± 14.50 | 320.38 ± 17.43 | 266.33 ± 20.07 | 265.81 ± 24.98 | 310.05 ± 27.26 | 298.72 ± 32.35 | 288.33 ± 23.75 | 284.98 ± 20.71 | 17.63 | 51.16 | 11.58 |
| BWG_{5-6}        | 541.21 ± 11.79 | 548.50 ± 8.94 | 475.46 ± 25.88 | 491.50 ± 19.14 | 443.46 ± 24.99 | 464.61 ± 34.65 | 491.25 ± 38.12 | 438.12 ± 27.66 | 416.71 ± 18.11 | 19.37 | 56.22 | 8.08 |
| BWG_{7-8}        | 539.00 ± 26.85 | 530.67 ± 18.07 | 490.77 ± 26.70 | 508.46 ± 32.79 | 489.04 ± 56.37 | 496.90 ± 27.26 | 501.51 ± 30.85 | 508.25 ± 33.95 | 487.29 ± 28.73 | 22.14 | NS | 8.75 |
| BWG_{9-0}        | 614.23 ± 15.99 | 525.71 ± 46.66 | 529.86 ± 24.31 | 504.42 ± 35.26 | 574.88 ± 19.89 | 566.94 ± 41.58 | 600.04 ± 8.29 | 621.76 ± 22.45 | 584.05 ± 42.99 | 31.29 | NS | 10.99 |

The means bearing different superscript within same row differ significantly from each other (p < 0.05)

Table 2: Mean weekly body weight gain (g) of broilers fed with different treatment diets

| Body weight gain | Treatments | T_1(Control) | T_2 | T_3 | T_4 | T_5 | T_6 | T_7 | T_8 | T_9 | SEM | CD at 5% | CV% |
|------------------|------------|--------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|------|------|
| BWG_{0-1}        | 125.79 ± 9.65 | 121.71 ± 5.72 | 116.58 ± 1.02 | 120.58 ± 3.00 | 117.34 ± 4.24 | 124.00 ± 4.75 | 116.69 ± 5.62 | 113.67 ± 3.73 | 108.29 ± 5.85 | 5.33 | NS | 9.02 |
| BWG_{1-2}        | 200.03 ± 7.94 | 166.71 ± 10.81 | 174.62 ± 14.50 | 194.58 ± 5.00 | 177.86 ± 13.53 | 205.99 ± 13.94 | 169.95 ± 16.06 | 160.68 ± 8.03 | 146.15 ± 10.63 | 11.37 | 33.00 | 12.82 |
| BWG_{3-4}        | 385.89 ± 3.17 | 319.00 ± 14.50 | 320.38 ± 17.43 | 266.33 ± 20.07 | 265.81 ± 24.98 | 310.05 ± 27.26 | 298.72 ± 32.35 | 288.33 ± 23.75 | 284.98 ± 20.71 | 17.63 | 51.16 | 11.58 |
| BWG_{5-6}        | 541.21 ± 11.79 | 548.50 ± 8.94 | 475.46 ± 25.88 | 491.50 ± 19.14 | 443.46 ± 24.99 | 464.61 ± 34.65 | 491.25 ± 38.12 | 438.12 ± 27.66 | 416.71 ± 18.11 | 19.37 | 56.22 | 8.08 |
| BWG_{7-8}        | 539.00 ± 26.85 | 530.67 ± 18.07 | 490.77 ± 26.70 | 508.46 ± 32.79 | 489.04 ± 56.37 | 496.90 ± 27.26 | 501.51 ± 30.85 | 508.25 ± 33.95 | 487.29 ± 28.73 | 22.14 | NS | 8.75 |
| BWG_{9-0}        | 614.23 ± 15.99 | 525.71 ± 46.66 | 529.86 ± 24.31 | 504.42 ± 35.26 | 574.88 ± 19.89 | 566.94 ± 41.58 | 600.04 ± 8.29 | 621.76 ± 22.45 | 584.05 ± 42.99 | 31.29 | NS | 10.99 |

The means bearing different superscript within same row differ significantly from each other (p < 0.05)
Table 3: Feed consumption (g/bird) of broiler fed with different treatment diets

| Feed consumption | Treatments | T1 (Control) | T2 | T3 | T4 | T5 | T6 | T7 | T8 | T9 | SEM | CD at 5% | CV% |
|------------------|------------|--------------|----|----|----|----|----|----|----|----|-----|--------|------|
| **FCO-1**        |            | 1.69 ± 0.09  | 1.75 | NS | 1.76 | 50.62 | 1.63 | 5.61 | 1.77 | 1.90 ± 0.02 | 7.04 | NS | 8.12 |
| **FC1-2**        |            | 3.68         | 4.97 | 1.73 | 11.68 | 2.67 | 5.43 | 8.50 | 5.73 | 11.28 | 0.04 | NS | 8.50 |
| **FC2-3**        |            | 39.84 ± 0.09 | 342.67 | 360.92 | 351.96 | 363.63 | 376.71 | 346.13 | 362.79 | 333.71 | 15.11 | NS | 10.89 |
| **FC3-4**        |            | 599.13 ± 0.09 | 540.00 | 569.09 | 576.63 | 538.33 | 565.79 | 592.71 | 529.84 | 536.83 | 30.54 | NS | 12.99 |
| **FC4-5**        |            | 889.09 ± 0.09 | 893.13 | 899.42 | 898.75 | 877.22 | 880.59 | 926.71 | 889.46 | 827.87 | 29.29 | NS | 6.61 |
| **FC5-6**        |            | 997.13 ± 0.09 | 975.79 | 890.67 | 928.25 | 887.67 | 907.71 | 876.51 | 936.13 | 854.42 | 59.59 | NS | 46.23 |
| **TFC0-1**       |            | 1.251.68 ± 0.09 | 1.197.38 | 1121.75 | 1120.50 | 1226.38 | 1089.25 | 1177.75 | 1248.38 | 1170.09 | 50.62 | NS | 8.59 |
| **TFC0-2**       |            | 156.59 ± 0.09 | 59.42 | 40.68 | 65.69 | 42.66 | 59.22 | 60.36 | 22.60 | 29.62 | 18.12 | NS | 6.85 |
| **TFC1-2**       |            | 353.15 ± 0.09 | 508.71 | 531.75 | 519.04 | 545.94 | 554.75 | 517.17 | 539.13 | 510.71 | 18.12 | NS | 5.11 |
| **TFC2-3**       |            | 1488.21 ± 0.09 | 1433.58 | 1468.50 | 1462.13 | 1451.55 | 1446.38 | 1519.97 | 1419.29 | 1364.70 | 46.99 | NS | 4.69 |
| **TFC3-4**       |            | 1420.25 ± 0.09 | 25.83 | 53.82 | 33.72 | 87.70 | 18.36 | 41.31 | 27.91 | 53.78 | 95.04 | NS | 9.07 |
| **TFC4-5**       |            | 2248.81 ± 0.09 | 2173.17 | 2012.42 | 2048.75 | 2114.04 | 1996.96 | 2053.75 | 2184.50 | 2025.50 | 119.92 | NS | 5.61 |
| **TFC5-6**       |            | 4268.52 ± 0.09 | 4115.46 | 4012.67 | 4029.92 | 4074.92 | 3998.09 | 4090.34 | 4142.92 | 3900.91 | 114.31 | NS | 5.61 |

The means bearing different superscript within same row differ significantly from each other (p < 0.05)

Table 4: Feed Conversion Ratio of broilers fed with different treatment diets

| FCR               | Treatments | T1 (Control) | T2 | T3 | T4 | T5 | T6 | T7 | T8 | T9 | SEM | CD at 5% | CV% |
|-------------------|------------|--------------|----|----|----|----|----|----|----|----|-----|--------|------|
| **FCR0-1**        |            | 1.01 ± 0.06  | 1.00 ± 0.04 | 1.05 ± 0.02 | 1.01 ± 0.08 | 1.12 ± 0.04 | 1.04 ± 0.01 | 1.05 ± 0.08 | 1.10 ± 0.01 | 1.14 ± 0.05 | 0.05 | NS | 9.53 |
| **FCR0-2**        |            | 1.44 ± 0.05  | 1.53 ± 0.07 | 1.59 ± 0.10 | 1.57 ± 0.06 | 1.47 ± 0.03 | 1.55 ± 0.05 | 1.69 ± 0.09 | 1.70 ± 0.22 | 0.07 | NS | 9.71 |
| **FCR0-3**        |            | 1.49 ± 0.05  | 1.61 AB ± 0.06 | 1.67 ± 0.04 | 1.75 ± 0.09 | 1.79 ± 0.06 | 1.63 ± 0.02 | 1.76 ± 0.08 | 1.76 ± 0.03 | 1.79 ± 0.06 | 0.05 | 0.17 | 6.92 |
| **FCR0-4**        |            | 1.55 ± 0.03  | 1.62 AB ± 0.03 | 1.77 ± 0.07 | 1.78 ± 0.08 | 1.87 ± 0.03 | 1.74 ± 0.03 | 1.82 ± 0.06 | 1.88 ± 0.04 | 1.87 ± 0.02 | 0.04 | 0.13 | 5.35 |
| **FCR0-5**        |            | 1.64 ± 0.04  | 1.69 ± 0.06 | 1.78 ± 0.03 | 1.79 ± 0.06 | 1.85 ± 0.03 | 1.77 ± 0.07 | 1.80 ± 0.07 | 1.86 ± 0.03 | 1.83 ± 0.04 | 0.05 | NS | 5.64 |
| **FCR0-6**        |            | 1.74 ± 0.04  | 1.83 ± 0.05 | 1.87 ± 0.04 | 1.90 ± 0.06 | 1.93 ± 0.03 | 1.81 ± 0.09 | 1.84 ± 0.06 | 1.90 ± 0.02 | 1.88 ± 0.03 | 0.05 | NS | 5.34 |

The means bearing different superscript within same row differ significantly from each other (p < 0.05)
The effect of dietary choline from different sources on the performance of commercial broiler chicken was studied. The results indicated that choline chloride (60% @ 1000 g/ton feed) and这首行中文内容...\n
The average weekly feed conversion ratio (FCR) recorded at each week, is presented in Table 4. The periodic FCR, including FCR_0.6 of birds fed with different diets did not differ significantly among each other, except at FCR_0.7 and FCR_0.4 phases. Better feed conversion ratio (1.74) was found in the birds fed with T_1 (Control) diet, whereas poorer FCR was presented in Table 6. The ROFC of birds fed with different treatment diets was found to be useful to improve the overall performance of broilers, as it showed higher body weight & body weight gain, better FCR and ROFC as compared to other rations with lower choline chloride or all levels of herbal choline and phosphatidyl choline. Total feed consumption and feed conversion ratio of birds fed with treatment rations supplemented with different choline sources were however at par for all rations. The mortality pattern of the birds was within the permissible limits and revealed no adverse effects on the feeding of choline from different sources in diet.

**Conclusion**

From the study, it may be concluded that the choline chloride 60% supplemented at dose level of @ 1000 g/ton of feed (control) was found to be useful to improve the overall performance of broilers, as it showed higher body weight & body weight gain, better FCR and ROFC as compared to other rations with lower choline chloride or all levels of herbal choline and phosphatidyl choline. Total feed consumption and feed conversion ratio of birds fed with treatment rations supplemented with different choline sources were however at par for all rations. The mortality pattern of the birds was within the permissible limits and revealed no adverse effects on the feeding of choline from different sources in diet.

**Acknowledgement**

The authors are grateful to the Dean of the Veterinary College...
and authorities of Anand Agricultural University, Anand for the fund and facilities provided for this research work.

REFERENCES
Chatterjee, S., & Misra, S.K. (2004). Efficacy of herbal biocholine in controlling fatty liver syndrome in commercial broiler on high metabolic energy diet. *Phytomedica*, 5, 37-39.

Farina, G., Kessler, A.D.M., Ebling, P.D., Marx, F.R., César, R., & Ribeiro, A.M.L. (2017). Performance of broilers fed different dietary choline sources and levels. *Ciência Animal Brasileira*, 18.

Ferguson, S.M., Bazalakova, M., Savchenko, V., Tapia, J.C., Wright, J., & Blakely, R.D. (2004). Lethal impairment of cholinergic neurotransmission in hemicholinium-3-sensitive choline transporter knockout mice. *Proceedings of the National Academy of Sciences*, 101(23), 8762-8767.

Fouladi, P., Nobar, R.S.D., & Ahmadzade, A. (2008). Effect of choline chloride supplement and canola oil on the performance and feed efficiency in the broiler chickens. *Research Journal of Poultry Science*, 3, 58-62.

Igwe, I.R., Okonkwo, C.J., Uzoukwu, U.G., & Onyenegecha, C.O. (2015). The effect of choline chloride on the performance of broiler chickens. *Annual Research and Review in Biology*, 8(3), 1-8.

Jadhav, N.V., Nagbhushana, V., Maini, S., & Kartikesh, S.M. (2008). An evaluation of comparative effects of feeding synthetic and herbal choline on broiler performance, nutrient balance and serum activities. *Veterinary World*, 1(10), 306.

Kathirvelan, C., Chandrasekaran, D., Vasanthakumar, P., & Purushothaman, M.R. (2013). Effect of replacement of synthetic choline with herbal choline on growth performance of broilers. *Indian Journal of Animal Nutrition*, 30(2), 184-187.

Khose, K.K., Manwar, S.J., Gole, M.A., Ingole, R.S., & Rathod, P.R. (2018). Efficacy of herbal choline as a replacement of synthetic choline chloride in diets on growth performance of broilers. *Journal of Livestock Research*, 8(10), 313-322.

Koujalagi, S., Chhabra, S., Randhawa, S.N.S., Singh, R., Randhawa, C.S., & Kashyap, N. (2018). Effect of herbal biocholine supplementation on oxidative stress and biochemical parameters in transition dairy cows. *The Pharma Innovation Journal*, 7(4), 842-847.

Kumar, P., & Sharma, S. (2014). Trial study comparing effect of cholmax (herbal choline) and synthetic choline chloride on growth performance in broiler chicken. *Indian Journal of Research*, 5(10), 200-202.

Selvam, R., Saravanakumar, M., Suresh, S., Chandrasekeran, C.V., & Prashanth, D.S. (2018). Evaluation of polyherbal formulation and synthetic choline chloride on choline deficiency model in broilers: implications on zootechnical parameters, serum biochemistry and liver histopathology. *Asian-Australasian Journal of Animal Sciences*, 31(11), 1795.

Sharma, A., & Ranjan, S. (2015). Effect of herbal and chemically synthetic choline on physio-biochemical characteristic of chicks. *Journal of Global Bioscience*, 4(6), 2537-2542.

Snedecor, G.W., & Cochran, W.G. (1994). *Statistical Methods* (8th ed.). Oxford & IBH Publishing Co. Calcutta, India.

Wen, Z.G., Tang, J., Hou, S.S., Guo, Y.M., Huang, W., & Xie, M. (2014). Choline requirements of white pekin ducks from hatch to 21 days of age. *Poultry Science*, 93(12), 3091-3096.

Zhang, C.X., Pan, M.X., Li, B., Wang, L., Mo, X.F., Chen, Y.M., & Ho, S.C. (2013). Choline and betaine intake is inversely associated with breast cancer risk: A two-stage case-control study in China. *Cancer Science*, 104(2), 250-258.