# FUNCTIONAL POLYMORPHISMS AT A CANDIDATE GENE FOR MEAT AND EGG PRODUCTION IN INDIGENOUS CHICKENS OF KENYA

***B. P. Aswani[1], J. Kasiiti[2], P. Oyier[3], S. Maina[4], M. Makanda[5], L. Wamuyu[6], A. Alakonya[7], N. Kisa[8] and S. Ommeh[9]***

*[1, 5, 6, 7,9]Institute for Biotechnology Research, Jomo Kenyatta University of Agriculture and Technology*

*[1, 2, 8] State Department of Veterinary Services, Ministry of Agriculture, Livestock and Fisheries*

*[3,4]Department of Information Technology, Jomo Kenyatta University of Agriculture and Technology*

**Abstract**

Indigenous chickens provide large proportion of quality protein in human diet in terms of meat and eggs. Indigenous chicken are reared under the free range system. Commercial chickens produce much more meat and eggs; but the meat and egg quality has been affected in terms of taste, flavor and micronutrients. Indigenous chickens are largely unexploited and have ecotypes capable of producing more meat and eggs. Cross-breeding of indigenous chickens with exotic chickens to increase meat and egg production has led to genetic erosion thus loss of important production traits. This research characterized functional polymorphisms of two candidate genes associated with meat and egg production in the indigenous chicken. DNA was extracted from 96 unrelated chickens sampled from three populations not involved in the initial cross-breeding improvement program: TransNzoia, West Pokot, Turkana, Marsabit and Lamu counties. The presence of Prolactin gene and Insulin growth like factor 1 gene were amplified using an optimized PCR protocol. Electrophoresis was then carried out on the PCR products on a 2% Agarose gel and the bands viewed under a UV light. The band size for prolactin gene was found to be 130bp and 154 bp while for IGF-1 gene was found to be 813 bp .These two genes were sequenced and polymorphisms identified. Prolactin gene was found to have a 24bp INDEL while IGF-1 was found to have point mutations and INDEL's. This is the first study in Kenya to sequence production traits genes of indigenous chickens. Analysis of the production traits will be used for molecular breeding work and be adopted by the national poultry breeding programs to increase quality meat and egg production in indigenous chicken.

**Key words**: Breeding programs, ecotypes, food security, production systems, poultry, traits

## 1.0 Introduction

Indigenous chicken is one of the most important livestock worldwide because it provides a large proportion of protein in human diet in terms of eggs and meat. Chickens have also been used for ornamental purposes (e.g. silkie or bantams) and entertainment (e.g. gamecocks used for cock fighting) (Magothe et al 2012). In the Kenyan census which was carried out in 2009, results showed that the population of poultry is about 32 million and out of these, almost 70% are indigenous chicken whereas the commercial and hybrid constitute about 20% (Oparanya 2010), the remaining 10% comprises the other poultry such as turkeys, ducks, geese, quails, ostrich, pigeons and guinea fowls FAO (2007).Most of the indigenous chicken are kept by the majority rural poor to fulfill multiple functions among them supply of cheap affordable protein in their nutrition and source of income FAO (2007).

Several candidate genes associated with egg production in chicken have been studied and they include; gonadotrophin releasing hormone-I (*GnRH-I*), neuropeptide Y (*NPY*), dopamine D2 receptor (*DRD2*), vasoactive intestinal polypeptide (*VIP*), VIP receptor-1 (*VIPR-1*), and prolactin (*PRL*). Among these genes prolactin (*PRL*) has been widely studied giving room for comparative study. As compared to the other genes it has been found to have a strong association with meat production, since they are polygenic traits some genes have got a strong effect than others as in the case of *PRL*.

Prolactin gene promoter is highly polymorphic, and has significant effects on egg quality traits in White Leghorn hens (Liu *et al*., 2010). The elevation of egg production and the inhibition of incubation behavior are the aims of modern poultry production. Prolactin (*PRL*) gene is confirmed to be critical for the onset and maintenance of these reproductive behaviors in birds. The prolactin gene promoter is highly polymorphic, and has significant effects on egg quality traits in White Leghorn hens (Bhattacharya *et al*., 2011).

Several candidate genes associated with meat production in chicken have been studied and they include: *Spot14α* gene, *Stat5B* gene, Insulin-like growth factor binding protein1 (*IGFBP1*), Insulin-like growth factor binding protein 2 (*IGFBP2*), Insulin-like growth factor binding protein 3(*IGFBP3*) and Insulin-like growth factor I (*IGF-I)*. Among these genes Insulin-like growth factor I (*IGF-I)* has been widely studied giving room for

comparative study. As compared to the other genes it has been found to have a strong association with meat production, since they are polygenic traits some genes have got a strong effect than others as in the case of *IGF-I*. Insulin-like growth factors (IGF) consist of a family of polypeptide hormones structurally associated with insulin with multiple metabolic and anabolic functions (Zhou *et al*., 2005).
The IGF-I stimulate the proliferation, differentiation, and metabolism of myo-genic cell lines from different species. The IGFs have been shown to regulate body and muscle growth in chicken. The *IGF1* gene may play important roles in growth of multiple tissues, including muscle cells, cartilage, and bone. Chickens with high circulating IGF-1 mRNA levels have got a high growth rate line (Zhou *et al*., 2005).

## 2.0 Materials and Methods

### 2.1 Study Area

This study was conducted in TransNzoia, West Pokot, Turkana, Marsabit and Lamu counties. These were the preferred study sites since some of these areas were not adversely affected by the cockerel and pullet exchange programme. (**Table 1**)

*Table 1: Summary of sampled locations*

| County | Number of samples |
|---|---|
| TransNzoia | 13 |
| West Pokot | 18 |
| Turkana | 10 |
| Marsabit | 23 |
| Lamu | 32 |
| **TOTAL** | ***96*** |

### 2.2 Sample Collection

Blood samples were collected from 96 genetically unrelated chickens and stored at JKUAT metadata base from Lamu, Turkana, TransNzoia, West Pokot, Marsabit counties and reference populations. Blood was collected from the wing vein of chickens and spotted on FTA cards. Genomic DNA was extracted from air dried blood preserved on FTA classic cards (Whatman Biosciences) using the manufacturers' protocol. The blood was drawn from several populations. Each population comprised of 24-32 individuals per population following the recommendations of (Hale *et al*., 2012).Two mature birds were sampled per flock and the sampling strategy and characteristics of the sampling locations was employed.

### 2.3 Pcr Amplifications

Reverse and forward primers were designed based on the SNP's of the candidate genes. . The relevant PCR primers (forward: 5' -CATTGCGCAGGCTCTATCTG-3'; reverse: 5' reverse: 5'TCAAGAGAAGCCCTTCAAG-3') and Prolactin gene the primers used were forward primer TTTAATATTGGTGGGTGAAGAGACA, reverse primer ATGCCACTGATCCTCGAAAACTC. PCR amplifications was carried out in 25 µl reaction volumes containing 20 ng genomic DNA, 2 X PCR buffer (100 mM Tris–with 0.1% SDS at pH 8), 2.5 mM of each dNTP, 20 pM of each primer and 1 unit of dream Taq Green Master Mix 2X (Promega, Madison WI, USA). Thermocycling conditions were; $94^0$C (3 min), 35 cycles of $94^0$C (1 min), $58^0$C (1 min), $72^0$C (2 min) and a final extension step at $72^0$C (10 min).

### 2.4 PCR Purification

PCR products were purified using the Wizard SV Gel and PCR Clean-Up Kit (Promega, Madison WI, and USA). Purified products were sequenced directly.

### 2.5 DNA Sequence Analysis

Direct sequencing was done using the Sanger technique that was able to amplify up to 1000bp. Chromas Lite version 2.1 (2012), was used to Edit the sequences and CLUSTAL X (2.0) was used to perform the alignment.

### 2.6 Ethical Clearance

This study got a no objection for the research under the permit number "RES/POL/VOL.XXVII/162" from the Ministry of Agriculture, Livestock and Fisheries state department of veterinary services.

**3.0 Results**

**3.1 Insulin-Like Growth Factor 1 Gene Candidate Gene for Meat Production in Chicken**

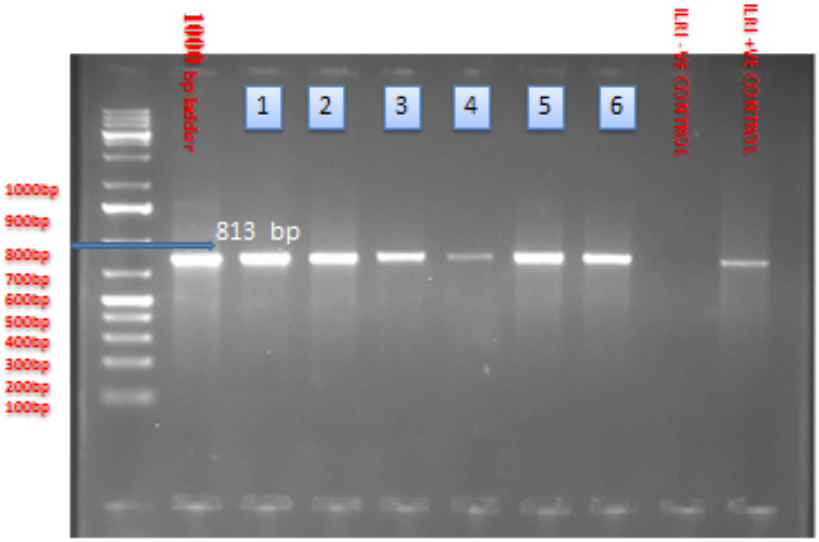


**Figure 1: PCR amplification gel image of the Insulin-like growth factor 1 gene of chicken. Lane 1-6 this are the chicken samples.**

The amplified product was 813bp an indication that the amplicon was IGF-1.This has been shown to be the case in different studies that showed the same size for igf-1 (Zhou *et al*., 2005).

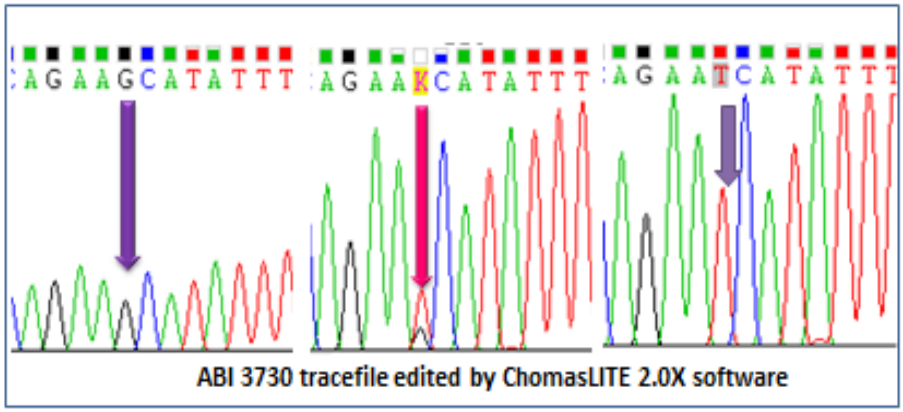


**Figure 2 : Polymorphism in the sequence chromatograms of IGF-1 gene. Shown by substitution mutation of G and T at 230bp.**

The chromatograms upon editing manually using Chromas Lite 2.0 X software a point mutation of G and T was found and this was denoted by K according to the software. It was a SNP that showed evidence of polymorphism in the IGF-1 gene as shown in other studies (Eman M. Goudaa and Gamal S. Essawyb 2010)

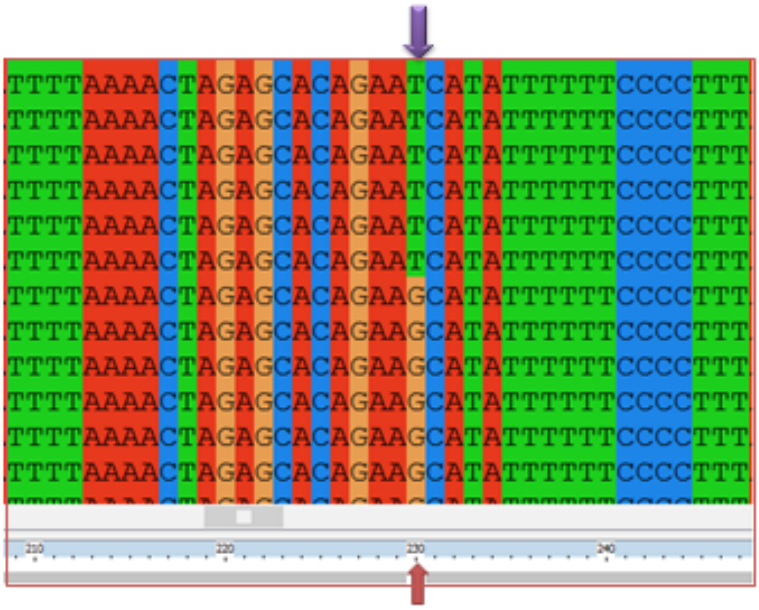

**Figure 3:Multiple sequence alignment showing the substitution point mutation of G and T at 230 base pair. Clustal X version 2.1.**

Upon alignment using Clustal X software this confirmed the presence of the point mutation at 230 bp.

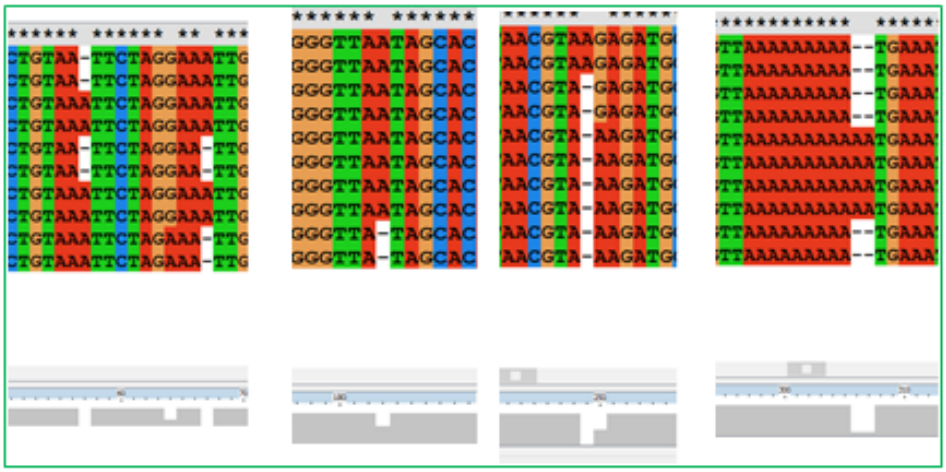

**Figure 4:Multiple sequence alignment showing insertion deletion mutations along the iGF-1 gene in chicken**

There were several insertion deletion mutations along the IGF-1 gene as shown in figure 4 above indicating polymorphisms in the gene.

### 3.2 Prolactin Gene Candidate Gene for Egg Production in Chicken

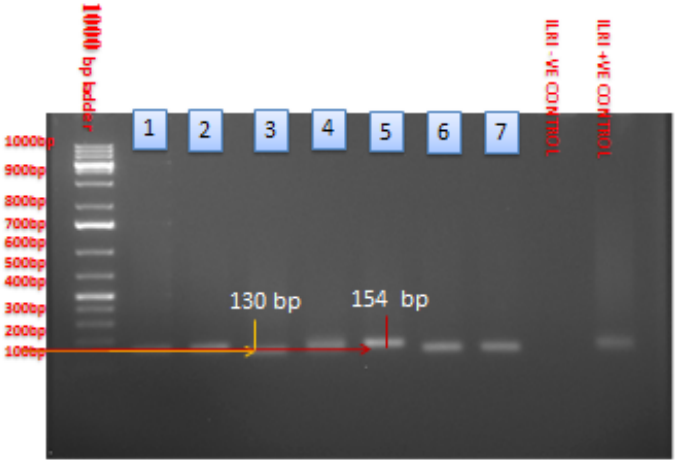


**Figure 5: PCR amplification gel image of the prolactin gene of chicken. Lane 1-7 this are the chicken samples.**

The amplified products were 130bp and 154bp an indication that the amplicon was prolactin gene. The gene had a 24 bp insertion deletion mutation as it has been shown to be the case in different studies that showed the same size for prolactin (Liu *et al*., 2010).

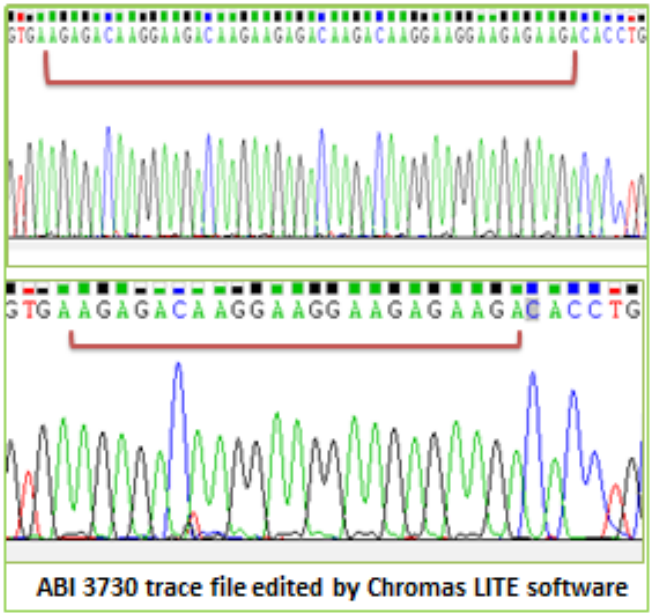


**Figure 6 : Polymorphism in the sequence chromatograms of PRL gene. Shown by insertion deletion mutation of 24bp.**

The chromatograms showed 24bp insertion deletion mutations along the PRL gene a suggestive indication of polymorphisms in the gene.

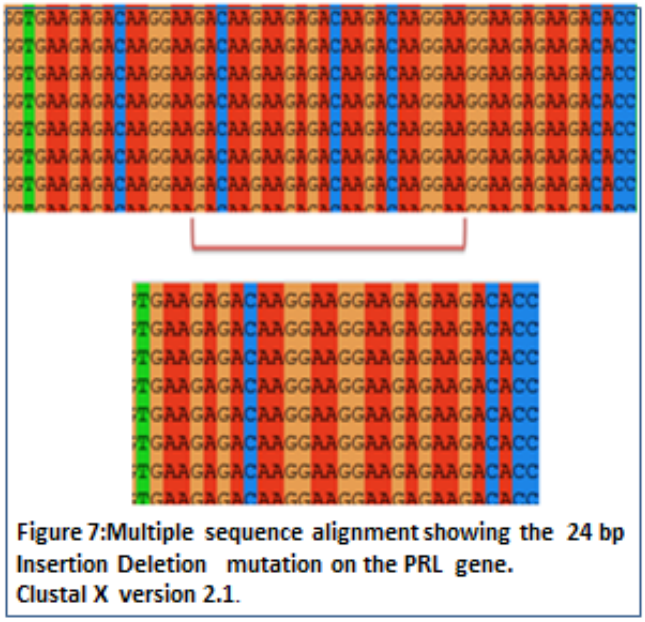

Figure 7:Multiple sequence alignment showing the 24 bp Insertion Deletion mutation on the PRL gene. Clustal X version 2.1.

The 24 bp Insertion Deletion mutation was seen after the multiple alignment of the sequences indicating polymorphisms

## 4.0 Discussions

### 4.1 IGF-1 GENE

Amplicons of the expected sizes were obtained from the different chicken DNA samples. These were approximately 813 bp in size as predicted by the published chicken sequence (Fig. 2). The amplicons exhibited polymorphism upon sequencing, editing and analysis of the sequences evidently shown by the chromatograms and alignments (Fig 2, 3, 4) .The SNP's were well distributed in the populations sampled an indication that IGF-1 gene is a highly polymorphic gene (Zhou *et al*., 2005).

Lamu county had the highest point mutations of G/T of 55% of the population while TransNzoia had the lowest point mutations of 36% of the population, West Pokot, Turkana and Marsabit had 43%,40%,395 respectively G/T point mutations. While the distribution of the insertion deletion mutations were on a half to half distribution among the different counties.

### 4.2 PRL Gene

Amplicons of the expected sizes were obtained from the different chicken DNA samples. These were approximately 130 bp and 154 bp in size as predicted by the published chicken sequence (Fig. 5). The amplicons exhibited polymorphism upon sequencing, editing and analysis of the sequences evidently shown by the chromatograms and alignments (Fig 6, 7)(Liu *et al*., 2010;Cui et al 2006).

All the counties had a high number of 24 bp Insertion mutation with Lamu having the highest number of 65.6% and Turkana the lowest with 3.2% .The deletion mutation was low and Lamu had only 18.75% while Marsabit and TransNzoia had 9.3%.

From the research it may be stated that prolactin gene is highly polymorphic and the SNP's are the functional polymorphisms for egg production traits.(Bhattacharya *et al*., 2011)

## 5.0 Conclusion

The study was able to assess polymorphisms on the IGF-1 gene as the candidate gene with increased meat production in indigenous chickens in Kenya. The study was also able to assess polymorphisms on the prolactin gene as the candidate gene with increased egg production in indigenous chickens in Kenya.

## 6.0 Recommendations

The polymorphisms obtained at the candidate genes will be useful markers that can be adopted and implemented by the national poultry breeding programs to design a selective molecular breeding tool. This approach should select for high meat and egg producing indigenous chickens hence in the long run will enable food security. Findings from this research should direct further research to investigate all of the above factors.

**Acknowledgment**
The authors wish to acknowledge financial support awarded to Dr. Sheila Ommeh by the Jomo Kenyatta University of Agriculture and Technology research grant no. JKU/2/4/RP/181.We also acknowledge The National Commission for Science, Technology and Innovation grant number NACOSTI/RCD/ST&I 5TH CALL MSC/005.We would also like to thank the Department of Veterinary Services for supporting this research. Appreciation is also extended to the extension officers and the farmers for their co-operation during data collection.

**References**

Bhattacharya, T. K., R. N. Chatterjee, R. P. Sharma, U. Rajkumar, M. Niranjan, and B. L. N. Reddy,( 2011). "Association of Polymorphism in the Prolactin Promoter and Egg Quality Traits in Laying Hens." *British Poultry Science*52 (5): 551–557.

Chromas Lite version 2.1 (2012), Technelysium Pty Ltd, South Brisbane, Queensland, Australia.

Cui, J. X., Du, H. L., Liang, Y., Deng, X. M., Li, N., & Zhang, X. Q. (2006). Association of polymorphisms in the promoter region of chicken prolactin with egg production. *Poultry science*, *85*(1), 26-31.

Fayemi P. O. and V. Muchenje (2012)" Meat in African context: From history to science" *African Journal of Biotechnology* 11(6), 1298-1306,( January)

http://www.academicjournals.org/AJB DOI: 10.5897/AJB11.2728.ISSN 1684–5315 © Academic *Journals Review*

FAO,(2012)."Phenotypic characterization of animal genetic resources". FAO Animal Production and Health Guidelines No. 11. Rome http://www.fao.org/docrep/015/i2686e/i2686e00.pdf

FAO, (2007) "The structure and importance of the commercial and village based poultry systems in Kenya" FAO Poultry report sector No. 23 Rome.

Gouda, E. & Essawy, G. (2014). Polymorphism of Insulin-Like Growth Factor I Gene among Chicken Breeds in Egypt. *Zeitschrift für Naturforschung C*, 65(3-4), pp. 284-288. Retrieved 11 Oct. 2015, from doi:10.1515/znc-2010-3-418

Granevitze Z, Hillel J, Chen GH, Cuc NT, Feldman M, Eding H, Weigend S.( 2007 ) "Genetic diversity within chicken populations from different continents and management histories": *Animal Genetics* :38(6):576-83

Hillel J, Groenen MA, Tixier-Boichard M, Korol AB, David L, Kirzhner VM, Burke T, Barre-Dirie A, Crooijmans RP, Elo K, (2003) "Biodiversity of 52 chicken populations assessed by microsatellite typing of DNA pools" *Genet Sel Evol. Sep-Oct; 35(5):533-57.*

Hale ML, Burg TM, Steeves TE, (2012). "Sampling for Microsatellite-Based Population Genetic Studies: 25 to 30 Individuals per Population Is Enough toEstimate Allele Frequencies". PLoS ONE 7(9): e45170. doi:10.1371/journal.pone.0045170

Kingori A.M, A.M. Wachiraand J.K. Tuitoek (2010) "Indigenous Chicken Production in Kenya": A Review. *International Journal of Poultry Science*9 (4): 309-316, 2010ISSN 1682-8356© Asian Network for Scientific Information, C309

Larkin,M.A., Blackshields, G., Brown, N.P., Chenna, R., McGettigan, P.A., McWilliam, H., Valentin, F., Wallace, I.M., Wilm, A., Lopez, R., Thompson, J.D., Gibson, T.J., Higgins, D.G. (2007) Clustal W and Clustal X version 2.0. Bioinformatics, 23:2947-2948.

Liu, W. J., D. X. Sun, Y. Yu, G. Li, S. Q. Tang, Y. Zhang, and Y. C. Wang,(2010). "Association of Janus Kinase 2 Polymorphisms with Growth and Reproduction Traits in Chickens." *Poultry Science*89 (12): 2573–2579.

Magothe, T. M., T. O. Okeno, W. B. Muhuyi, and A. K. Kahi, (2012). "Indigenous Chicken Production in Kenya: I. Current Status." *World's Poultry Science Journal* 68 (01): 119–132.

Messens, W., K. Grijspeerdt, and L. Herman. (2005)" Eggshell penetration by *Salmonella*" A review. *World's Poultry Science Journal*. 61:71–85.

Mui,r W. M., Wong, G.K., Zhang,Y., Wang,J., Martien, A. M., Groenen, Richard, P. M. A.,. Megens,H J Huanmin Zhang, Okimoto,R, Vereijken,A., Jungerius,A., Gerard,A.A., Lawley,C.T., Mary E. Delany, Sean MacEachern, and Cheng,H.H, (2008) "Genome-wide assessment of worldwide chicken SNP genetic diversity indicates significant absence of rare alleles in commercial breeds "*Proceedingsof the National Academyof Sciences of the United States of America"*105(45):17312-17317. doi:10.1073/pnas.0806569105

Mwacharo, J. M., G. Bjørnstad, V. Mobegi, K. Nomura, H. Hanada, T. Amano, H. Jianlin, and O. Hanotte,(2011). "Mitochondrial DNA Reveals Multiple Introductions of Domestic Chicken in East Africa." *Molecular Phylogenetics and Evolution*58 (2): 374–382.

Okeno, Tobias O, Alexander K Kahi, and Kurt J Peters, (2012a). "Characterization of Indigenous Chicken Production Systems in Kenya." *Tropical Animal Health and Production*44 (3) (March): 601–608. doi:10.1007/s11250-011-9942-x.

Okeno, Tobias O., Thomas M. Magothe, Alexander K. Kahi, and Kurt J. Peters,(2012). "Breeding Objectives for Indigenous Chicken: Model Development and Application to Different Production Systems." *Tropical Animal Health and Production* 45 (1): 193–203.

Zhou H, A. D. Mitchell, J. P. McMurtry, C. M. Ashwell, and S. J. Lamont. (2005)"Insulin-Like Growth Factor-I Gene Polymorphism Associations with Growth, Body Composition, Skeleton Integrity, and Metabolic Traits in Chickens1" *poultry science* 84:212-219.