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Comparative metagenomic analysis of gut microbial diversity of Indian native chicken (CARI Nirbheek) under different systems of management using NGS

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Abstract
Metagenomics is the culture independent analysis of microbial diversity in an ecosystem and it can be used to find out the gut microbial diversity of chicken. Native chicken is characterised by good genetic resistance and they are adapted to extensive system of management. NGS analysis of intestinal micro biome using primers targeting V_3, V_4 and V_4-V_6 region of 16S rRNA helped in revealing the microbial diversity of native chicken CARI Nirbheek. Comparison of gut microbial diversity under two management systems *viz*., intensive and extensive system was done using MG-RAST. *Lactobacillus* was the dominant genus under both systems of management. *Lactobacillus sakei* was the dominant bacterial strain under intensive system and *Lactobacillus helveticus* was dominant under extensive management. The present investigation helped in exploring the intestinal microbial diversity and identifying the bacterial strains which helps in increasing the genetic resistance and growth of native chicken under backyard rearing.

Keywords: Metagenomics, Illumina, MG-RAST, firmicutes and CARI Nirbheek

1. Introduction
The gastrointestinal micro biota has one of the highest cell densities for any ecosystem and in poultry ranges from 10^7 to 10^{11} bacteria per gram of gut content (Apajalahti *et al*., 2004) [1]. The majority of these microbes are uncharacterized and represent an enormous unexplored reservoir of genetic and metabolic diversity. The gut micro-biota has an important role in poultry health and production, which generally affects the health of the host by influencing digestion and nutrient absorption, intestinal morphology, and defence of the host against infection, Mead (2000) [2].
Metagenomics has been defined as function-based or sequence-based cultivation-independent analysis of the collective microbial genomes present in a given habitat (Riesenfeld *et al*., 2004) [3]. Metagenomics can be used to address the challenge of studying prokaryotes in the environment that are, as yet, unculturable and which represent more than 99% of the organisms in some environments (Amann *et al*., 1995) [4]. Recent advances in high throughput sequencing technologies have increased the number and size of metagenomic sequencing projects (Carola and Rolf, 2009) [5].
Bioinformatics tool like Meta Genomic Rapid Annotation using Subsystem Technology (MG-RAST) analysis provides a taxonomic classification and a new pipeline which computes results against many reference databases (GenBank, SEED, IMG, UniProt, KEGG and eggNOGs) (Meyer *et al*., 2008) [6].
Gut micro-biota is highly variable from individual to individual and also affected by several factors *viz.* environment, feed, genetic makeup of host etc. Native chicken are crosses of indigenous chicken developed for backyard system of rearing. They are adapted to extensive management system. There is no report on the whole gut microbial study of native chicken using culture independent methods. Metagenomic analysis of the gut micro-biome of native chicken will help in finding out the beneficial bacterial strains which enhance the growth and immunity of native chicken.

Keeping this in view, the present investigation was designed to find out the effect of rearing system on the gut microbial regime of CARI Nirbheek (cross of Aseel and Dahlem red) which have been developed and maintained at Desi unit of the institute.

2. Materials and Methods

All the experiments were conducted strictly in accordance with the guidelines of "Institutional Animal Ethics Committee" (IAEC). CARI Nirbheek is a native chicken developed at the Desi unit of the institute, CARI, Izatnagar. 10 chicks were reared under both management systems *viz*., intensively reared at experimental broiler farm, CARI, Izatnagar and extensively maintained under rural conditions at farmer's door about 15 km away from institute. Standard management conditions were followed under intensive system and under extensive management chicks were housed in kaccha houses made of locally available materials like asbestos sheet, card-board, mud etc. and fed on kitchen waste supplemented with broken grains and scavenging. The experiment was conducted during the month of December and February when ambient temperature ranged from 50.6 to 66.2°F and relative humidity 71-98%. Extensively reared birds showed a mortality percentage of 20.

Five chicks under each system of management were humanely slaughtered at 8 weeks age and whole intestine contents were collected and pooled aseptically. The gut contents were outsourced to M/s Genotypic Pvt Ltd., Bangalore India for Next Generation Sequencing. V_3, V_4, and V_4-V_6 hyper variable regions of 16srRNA were amplified using region specific primers and NGS was done using Illumina 300bp paired end platform. The data generated were analysed using bio-informatics software, MG-RAST, a fully automated service for annotation of metagenomic data.

2.1 Statistical analysis

Pearson's Chi-square ($\chi 2$) test (2×2 contingency) using SAS version 9.2 was employed for analysing significant differences in reads for CARI Nirbheek reared under extensive and intensive system under different phylogenetic taxa. The results have been presented in Table-1.

3. Results

3.1 Birds reared under intensive system of management

Quality check by MG-RAST filtered 90.2% of sequences and the remaining 34061 sequences represented the gut micro-flora using the V3, V4 and V4-V6 region of the bacterial 16S rRNA. Out of this 87.5% predicted to be protein coding. Sequence similarity searches are computed against a protein database derived from M5NR database. Remaining 12.5% of sequences hit against ribosomal RNA. Source hit distribution of 4257 sequences against Green genes could analyse 56.6% of sequences. SILVA LSU analysed only 0.006% of sequences and RDP could analyse 69.9% of sequences. 73.3% of sequences were analysed using SILVA SSU database. Taxonomic analysis was done using an E-value cut off of 1× 10-5, minimum identity cut off of 60% and minimum alignment length cut off of 15 amino acid.

3.2 Birds reared under extensive system of management

Quality check by MG-RAST filtered 1% of total sequences and the remaining 254058 sequences represented the gut micro flora using the V3, V4 and V4-V6 region of the bacterial 16S rRNA. Out of this 81.8% predicted to be protein coding. Sequence similarity searches were computed against a protein database derived from M5NR database. Remaining 18.2 of sequences hit against ribosomal RNA. Source hit distribution of 46238 sequences against Green genes analysed 60.82% of sequences. SILVA LSU analysed 0.02% of sequences and RDP could analyse 72.9% of sequences. 78.6% of sequences were analysed using SILVA SSU database. Taxonomic analysis was done using an E-value cut off of 1× 10-5, minimum identity cut off of 60% and minimum alignment length cut off of 15 amino acid.

3.3 Diversity of intestinal micro-biome at the level of various taxa

3.3.1 Phylogenetic profile at domain level

Under intensive management, Bacteria were the dominant domain accounting for (94.3%) of total micro-biome followed by Viruses (5%), Eukaryote (0.33%), others (0.13%) and Archaea (0.006%). Under extensive rearing, Bacteria were the dominant domain accounted for 92.33% of micro-biome followed by Viruses (7.03%), Eukaryote (0.3%), others (0.29%) and Archaea (0.022%). Domain level comparison of phylogenetic profile of CARI Nirbheek under different systems of rearing was given in Fig 1.

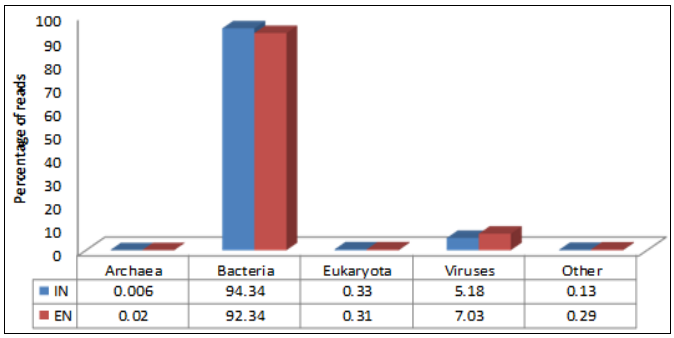

Fig 1: Domain level comparison of phylogenetic profile of CARI Nirbheek under different systems of rearing

3.3.2 Phylogenetic profile at phylum level

Under intensive system, phylum level analysis of sequences revealed that out of 38 phyla in total, 23 came under domain bacteria and among bacterial phyla, Firmicutes (76.8%) was dominant followed by Proteobacteria (7.2%), Actinobacteria (3.47%), Unclassified phyla derived from bacteria (3.2%) and Bacteroidetes (3.1%). Firmicutes / Bacteroidetes ratio for CARI Nirbheek under intensive management was 24.7. Minor phyla which constituted less than 1% of annotated reads were dominated by Tenericutes, Spirochetes, Cyanobacteria Fusobacteria and Chloroflexi. Only single phylum Euryarchaeotic (0.006%) formed domain archaea.

Under extensive system of rearing, total number of phyla was 37 and out of this27 phyla came under domain bacteria. Dominant phylum was Firmicutes (82.54%) followed by Actinobacteria (6.74%) and Proteobacteria (1.61%). Among minor phyla which were <1% of annotated reads, Bacteroidetes, Spirochaetes, Tenericutes, Cyanobacteria and Fusobacteria were dominant. Firmicutes/Bacteroidetes ratio for CARI Nirbheek under extensive. Phylum level comparison of phylogenetic profile of CARI Nirbheek under different systems of rearing was given in Fig 2.

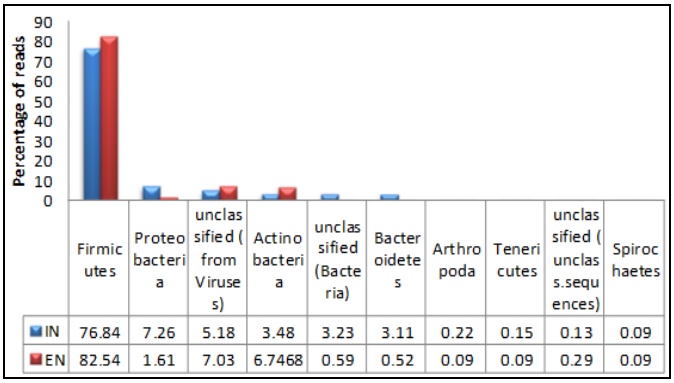

Fig 2: Phylum level comparison of phylogenetic profile of CARI Nirbheek under different systems of rearing

3.3.3 Phylogenetic profile at class level

Under intensive system, Bacilli (52.1%) formed dominant class followed by Clostridia (21.5%), Actinobacteria (3.4%), Epsilonproteobacteria (3.3%), Unclassified Class derived from Bacteria (3.2%), Bacteroidia (2.9%), Gammaproteobacteria (2%), Erysipelotrichi (1.6%), Deltaproteobacteria (1.6%), and Negativicutes (1.5%).

For Nirbheek under extensive management, Bacilli (76.96%), Actinobacteria (6.74%), Clostridia (4.39%), Gammaproteobacteria (1.05%), and Negativicutes (1.01%) were the major classes. Class level comparison of phylogenetic profile of CARI Nirbheek under different systems of rearing was given in Fig 3.

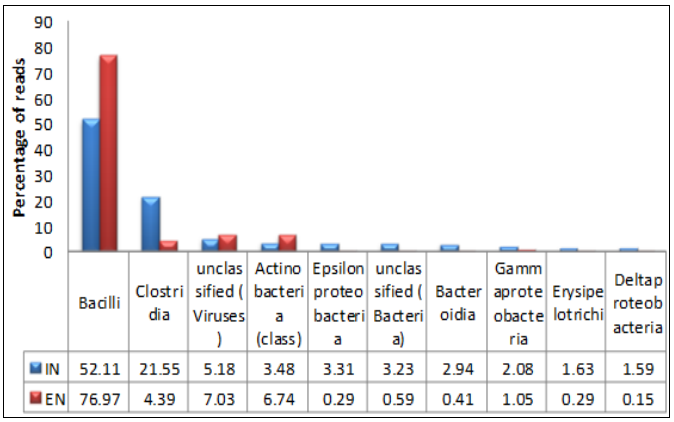

Fig 3: Class level comparison of phylogenetic profile of CARI Nirbheek under different systems of rearing was given

3.3.4 Phylogenetic profile at order level

Under intensive management, Lactobacillales (51.3%) and Clostridiales (21.1%) were dominant followed by Campylobacter ales (3.2%), unclassified orders derived from Bacteria (3.2%), Bacteroidales (2.9%), Coriobacteriales (2.2%), Enterobacteriales (1.9%), Erysipelotrichales (1.6%), and Selenomonadales (1.5%).

For Nirbheek under extensive system, Lactobacillales (76.14%) was the dominant order followed by Clostridiales (4.37%), Bifidobacteriales (4.11%), Coriobacteriales (1.76%) and Selenomonadales (1.01%). Order level comparison of phylogenetic profile of CARI Nirbheek under different systems of rearing was given in Fig 4.

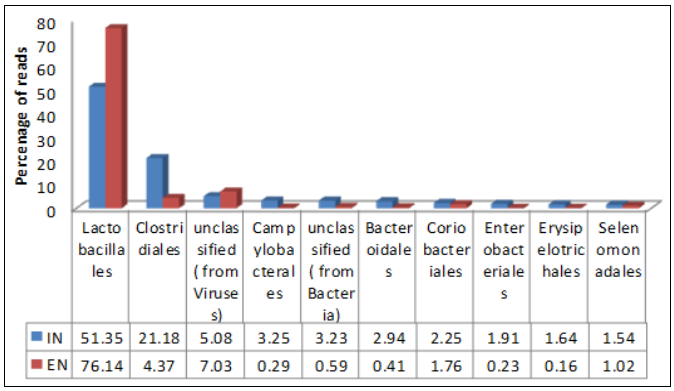

Fig 4: Order level comparison of phylogenetic profile of CARI Nirbheek under different systems of rearing was given

3.3.5 Phylogenetic profile at family level

Under intensive system of rearing, Lactobacillaceae (45.1%) was dominant followed by Ruminococcaceae (8.8%), Lachnospiraceae (5.1%), Enterococcaceae (4.7%), Unclassified family derived from Bacteria (3.2%), Clostridiaceae (2.9%), Campylobacteraceae (2.4%), Coriobacteriaceae (2.2%) and Enterobacteriaceae (1.89%).

For CARI Nirbheek, under extensive system, Lactobacillaceae (73.54%) was dominant followed by Bifidobacteriaceae (4.11%), Enterococcaceae (1.82%), Coriobacteriaceae (1.76%), Ruminococcaceae (1.4%), and Peptostreptococcaceae (1.05%). Family level comparison of phylogenetic profile of CARI Nirbheek under different systems of rearing was given in Fig 5.

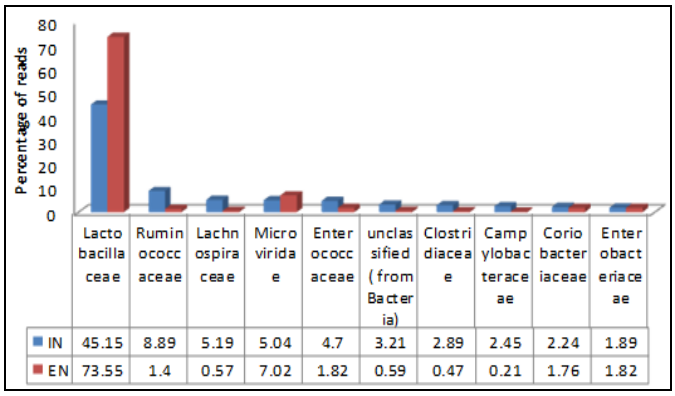

Fig 5: Family level comparison of phylogenetic profile of CARI Nirbheek under different systems of rearing was given

3.3.6 Phylogenetic profile at genus level

Under intensive system of rearing, dominant genera were *Lactobacillus* (45%) and *Faecalibacterium* (8.4%) followed by *Enterococcus* (4.6%), unclassified genus derived from Bacteria (3.2%), *Blautia* (2.76%), *Campylobacter* (2.44%), *Clostridium* (2.26%) and *Eubacterium* (1.72%).

Gut microbiome of CARI Nirbheek under extensive rearing was dominated by. *Lactobacillus* (73.44%) and *Bifidobacterium* (3.9%) followed by *Enterococcus* (1.68%), *Faecalibacterium* (1.05%) and unclassified genus derived from Peptostreptococcaceae (1.04%). Genus level comparison of phylogenetic profile of CARI Nirbheek under different systems of rearing was given in Fig 6.

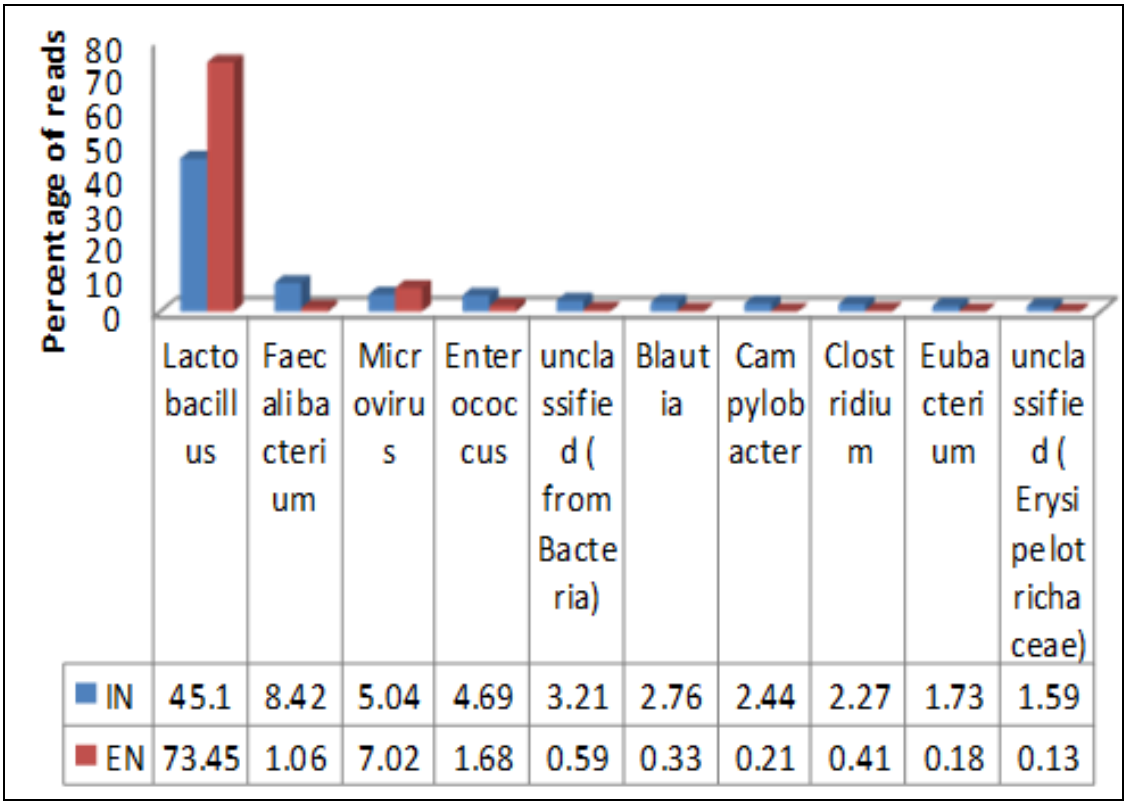

Fig 6: Genus level comparison of phylogenetic profile of CARI Nirbheek under different systems of rearing was given

3.4 Comparing the number of reads among different Phylogenetic taxa: The reads between the systems of management in CARI Nirbheek were significantly different (p<0.05) in each taxa; however the distribution/proportion of different entities under each taxa were different.

Table 1: Analysis of number of reads under different taxa for CARI Nirbheek between rearing systems

Management system	Phylogenetic Taxa					
	Domain					
	Bacteria	**Viruses**	**Others**	**Eukaryote**	**Archaea**	-
Intensive	271580[a]	14912[a]	389[a]	954[a]	19[a]	-
Extensive	190474[b]	14513[b]	637[b]	606[b]	46[b]	-
Phylum						
Intensive	Firmicutes	Actinobacteria	Proteobacteria	Bacteroidetes	Arthropoda	Cyanobacteria
	221330[a]	10015[a]	20916[a]	8974[a]	633[a]	123[a]
Extensive	170280[b]	13918[b]	3329[b]	1082[b]	193[b]	115[b]
Classes						
Intensive	Clostridia	Bacilli	Actinobacteria	Negativicutes	Gammaproteobacteria	Bacteroidia
	62073[a]	150099[a]	158785[a]	4439[a]	6013[a]	8486[a]
Extensive	9067[b]	158785[b]	13918[b]	2100[b]	2174[b]	855[b]
Order						
Intensive	Clostridiales	Lactobacillales	Coriobacteriales	Bacillales	Selenomonadales	Bacteroidales
	61015[a]	147905[a]	6508[a]	2194[a]	4439[a]	8486[a]
Extensive	9026[b]	157082[b]	3644[b]	1704[b]	2100[b]	855[b]
Family						
Intensive	Streptococcaceae	Lachnospiraceae	Ruminococcaceae	Enterococcaceae	Clostridiaceae	Veillonellaceae
	4136[a]	15053[a]	25782[a]	13639[a]	8401[a]	3893[a]
Extensive	1240[b]	1176[b]	2895[b]	3767[b]	975[b]	1710[b]
Genus						
Intensive	Lactococcus	Lactobacillus	Fecalibacterium	Enterococcus	Clostridium	Eubacterium
	3110[a]	130902[a]	24453[a]	13609[a]	6577[a]	5012[a]
Extensive	406[b]	151758[b]	2185[b]	3478[b]	845[b]	375[b]

Values having same superscripts in a column under each phylogenetic taxon between Management systems do not differ significantly (p<.05).

3.5 Top ranked ten species

The distribution of top-ranking species between CARI Nirbheek reared under intensive and extensive systems are presented in Table- 2. The top-ranking species in CARI Nirbheek reared under intensive system had the entities that are probiotic in nature involved in growth, better feed utilization and immunity besides some of the commensals which are normal inhabitant of intestine. For CARI Nirbheek under extensive system most of the dominant species were probiotic which are involved in immunity and growth.

Table 2: Taxon abundance of top ten species for CARI Nirbheek under two rearing systems

Species (IN)	Taxon abundance	Species (EN)	Taxon abundance
Lactobacillus sakei	116403	*Lactobacillus helveticus*	29091
Faecalibacterium prausnitzii	24453	*Lactobacillus delbrueckii*	19107
Enterobacteria phage phiX174 sensulato	14632	*Lactobacillus reuteri*	18715
Uncultured bacterium	8904	*Lactobacillus mucosae*	16711
Enterococcus faecalis	7620	*Enterobacteria* phage phiX174 sensulato	14509
Campylobacter avium	6261	*Lactobacillus pontis*	11738
Blautia sp. Ser8	5589	*Lactobacillus acidophilus*	7668
Clostridium scindens	3487	*Lactobacillus johnsonii*	6471
Escherichia coli	3451	*Lactobacillus vaginalis*	5012
Collinsella aerofaciens	3217	*Lactobacillus frumenti*	3668

4. Discussion

4.1 Analysis of gut micro-biome of CARI Nirbheek under two rearing systems

Statistical analysis for number of reads under various taxa revealed significant differences between two management systems. Many probiotic strains have been identified from gut micro--biome of CARI Nirbheek. *Lactobacillus helveticus* is a potential probiotic and modulate host immune response as reported by Borchers *et al*., (2009) [7] and Lebeer *et al*., (2010) [8]. Huang *et al*., (2004) [9] reported that *Lactobacillus casei* is a potential probiotic which helps in enhancing production performance and immunity.

Lactobacillus plantarum TN627 is a promising probiotic candidate with high potential for application as a supplement in the animal feed industry (Bejar *et al*., 2011) [10] and *Lactobacillus jensenii* used as immunobiotic (Villena *et al*., 2012) [11]. *Lactobacillus salivarius* found in extensively reared CARI Nirbheek act as probiotic and help in *Salmonella* reduction in poultry by competitive exclusion (Zhang *et al*., 2007) [12]. *Lactobacillus jensenii* which is used as immunobiotic (Villena *et al*., 2012) [11], has also been found in extensively reared CARI Nirbheek. *Bacteroides fragilis*, used as a potential probiotic and immunobiotic is reported from intensively reared CARI Nirbheek. Scupham *et al*. (2010) [13] reported that *Megamonas hypermegale* which belongs to phylum Firmicutes is a beneficial bacteria and recent metagenomics work revealed possible association between the presence of a subspecies of *Megamonas hypermegale* and *Campylobacter* suppression. It is also present in intensively reared CARI Nirbheek.

Thus, results of our experiment revealed that percentage of bacterial strains with probiotic properties were more in gut micro-biome of CARI Nirbheek and this could be contributing to the higher genetic resistance of native chicken in extensive or semi-intensive system of rearing. These beneficial bacterial strains can be isolated and used as a potential probiotic for broiler as reported by Musikasang *et al*. (2012) [14].

5. Conclusion

Analysis of gut micro biome of CARI Nirbheek under different rearing systems revealed that composition and profile of intestinal micro-biota are affected by management system. Chi -square analysis for the number of reads under various taxa revealed that the gut micro biome of CARI Nirbheek differs significantly under different rearing systems.

6. References

1. Apajalahti JHA, Kettunen A, Graham H. Characteristics of the gastrointestinal microbial communities, with special reference to the chicken. World's Poult Sci J. 2004;60:223–232.
2. Mead GC. Prospects for 'competitive exclusion' treatment to control salmonellas and other foodborne pathogens in poultry. Vet J. 2000;159(2):111-123.
3. Riesenfeld CS, Goodman RM, Handelsman J. Uncultured soil bacteria are a reservoir of new antibiotic resistance genes. Environ Microbiol. 2004;6(9):981-989.
4. Amann RI, Ludwig W, Schleifer KH. Phylogenetic identification and in situ detection of individual microbial cells without cultivation. Microbiol Rev. 1995;59(1):143-169.
5. Carola S, Rolf D. Achievements and new knowledge unravelled by metagenomic approaches. Appl Environ Microbiol. 2009;85:265–276.
6. Meyer F, Paarmann D, D'Souza M, Olson R, Glass EM, Kubal M, *et al*. The metagenomics RAST server – a public resource for the automatic phylogenetic and functional analysis of metagenomes. BMC Bioinformatics. 2008;9:386.
7. Borchers AT, Selmi C, Meyers FJ, Keen CL, Gershwin ME. Probiotics and immunity. J Gastroenterol. 2009;44:26–46.
8. Lebeer S, Vanderleyden J, De Keersmaecker SCJ. Host interactions of probiotic bacterial surface molecules: comparison with commensals and pathogens. Nat Rev Microbiol. 2010;8:171–184.
9. Huang MK, Choi YJ, Houde R, Lee JW, Lee B, Zha X. Effects of lactobacilli and an acidophilic fungus on the production performance and immune responses in broiler chickens. Poult Sci. 2004;83(5):788-795.
10. Beja W, Smaoui S, Makni M, Mellouli L, Bejar S. Biotechnol Bio process Eng. 2011;16:1120-1123.
11. Villena J, Suzuki R, Fujie H, Chiba E, Takahashi T, Shimazu T. Immunobiotic Lactobacillus jensenii modulates toll-like receptor 4-induced inflammatory response via negative regulation in porcine antigen presenting cells. Clin Vaccine Immunol. 2012;15:1038–1053.
12. Zhang G, Ma L, Doyle MP. Salmonellae reduction in poultry by competitive exclusion bacteria Lactobacillus salivarius and *Streptococcus cristatus*. J Food Prot. 2007;70(4):820-1053.
13. Scupham AJ, Jones JA, Weber TE. Antibiotic manipulation of intestinal microbiota to identify microbes associated with Campylobacter jejuni exclusion in Poultry. Appl Environ Microbiol. 2010;76(24):8026–8032.
14. Musikasang H, Tani A, H-kittikun A, Maneerat S. Probiotic potential of lactic acid bacteria isolated from chicken gastrointestinal digestive tract. World J Microbiol Biotechnol. 2009;25:1337–1345.