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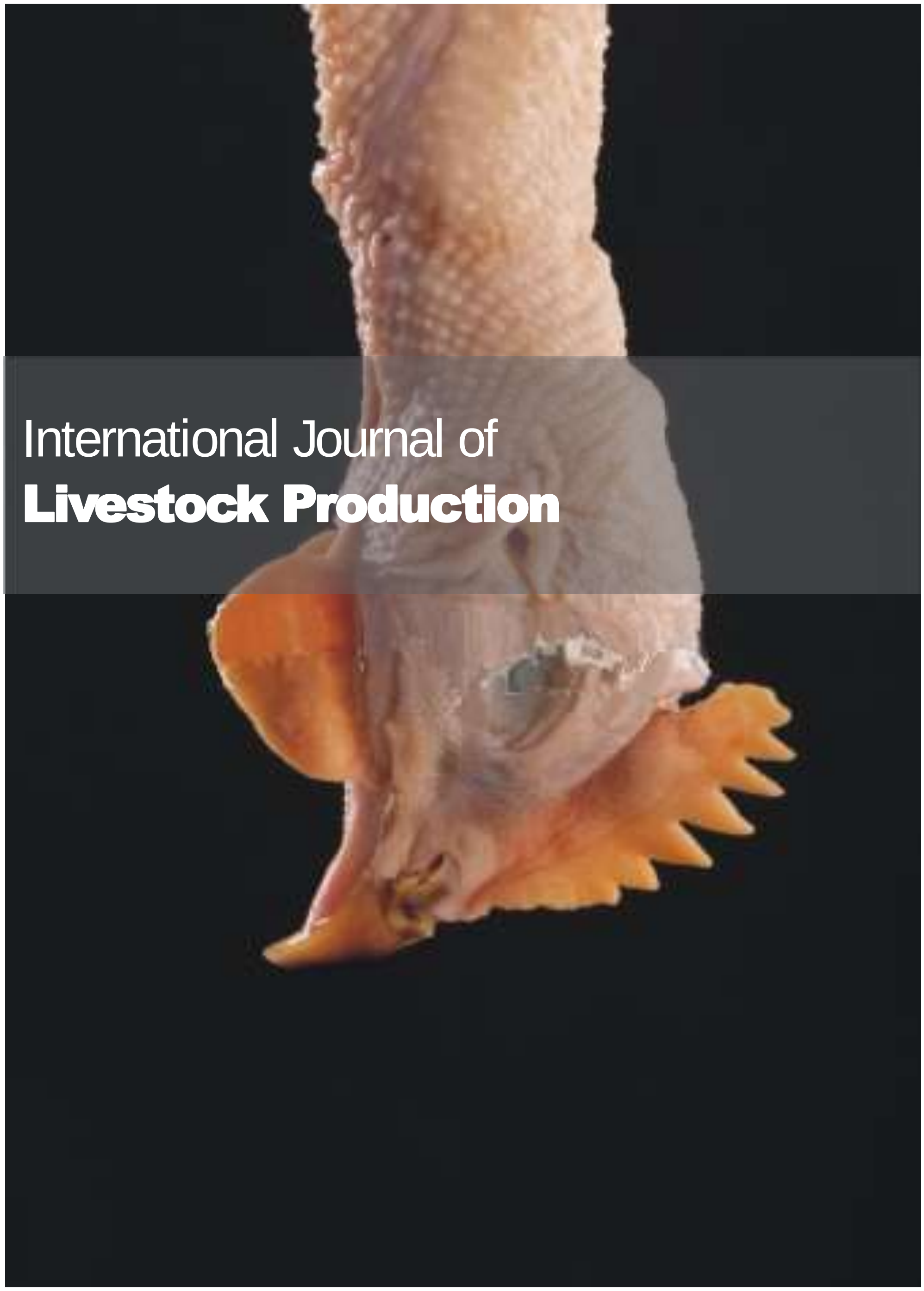

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Review

Cattle crossbreeding for sustainable milk production in the tropics

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Crossbreeding has been used to improve milk production performance of local cattle in the tropics. Crossbreeding exploits additive and non-additive allele gene effects leading to improvements in lactation length, decrease in calving interval, higher milk yields and early age of calving of cows and potentially increasing producer incomes. Varying levels of success have been reported for various crossbreeding programmes and the objective of the current review was to document the key challenges, best practices, lessons learnt and to propose sustainable interventions for future initiatives. Although crossbreeding has had some impacts on smallholder dairy production in the tropics, a number of bottlenecks affect its smooth implementation including inadequate funding, inappropriate policies, low participation of farmers and genotype and environment mismatches. The availability of large base of adapted local cattle genetic resources, innovative state of the art breeding technologies and goodwill of governments to make favourable policies and increase budgetary allocations for the livestock sector offer some prospects for crossbreeding for a sustainable dairy industry. Provision of the required infrastructure for improved management of crossbred dairy cattle including feeding and health care, access to markets, training of stakeholders, a well-trained and motivated local extension service personnel are recommended to help achieve this objective.

Key words: Artificial insemination, genotype-environment interaction, local breeds.

INTRODUCTION

The African Union Inter Bureau for Animal Resources (AUIBAR) in its Livestock Development Strategy for Africa (LiDeSA) 2015-2030 projects that per capita annual consumption of livestock products in Africa will increase 2 to 8-fold in the next two decades (AUIBAR, 2016). Already, total consumption of milk in developing countries has been projected to increase from 164 million metric tons in 1993 to 391 million metric tons this year (2020): a 138% increase while the expected increase in per capita consumption will be from 38 to 62 kg/person due to population increase, income growth and urbanization (Delgado, 2005; FAO, 2015; FAO, 2012; AUIBAR, 2019). Milk and dairy foods provide important nutrients such as calcium, magnesium and vitamin D that are beneficial to human life (Givens, 2018). With an expected increase in human population estimated at 100% for Africa by 2050 (UNDP, 2015), food insecurity will be a major problem unless food production is increased (Garcia et al., 2020). The local milk industry has the potential to help alleviate malnutrition among

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vulnerable groups through optimized production, processing and marketing channels (Parry-Hanson Kunadu et al., 2019). Low milk production in most tropical countries is due mainly to the use of local cattle breeds (FAO, 2010) which have not been selected for high milk production. There is a ten times difference in milk output per cow, from approximately 500 kg per annum in Africa and India to over 5000 kg in North America and Europe. Consequently, production to a higher level to meet the increasing demand of livestock products is limited using local cattle breeds motivating smallholder farmers to keep larger herds of cattle to produce higher amounts of milk (Thornton, 2010; FAO, 2010). At the same time, there is the major challenge of pasture unavailability to feed such large herds (FAO, 2013).

Although tropical cattle breeds are not genetically advantaged in terms of milk production, there is ample evidence of their adaptation and resistance to most endemic diseases and harsh climatic conditions. Therefore, to ensure an improvement in their sustainable productivity and also improving fitness characteristics to cope with the production environment strategic crossbreeding has been undertaken (Aboagye, 2014; Kebede et al., 2018) in many parts of sub-Saharan Africa. Crossbreeding exploits additive and non-additive allele gene effects leading to improvements in lactation length, decrease in calving interval, higher milk yield and early age of calving of cows and thus increase producer income. Crossbreeding has thus been employed as a tool for increased milk production in many countries in the tropics to produce crossbreds which are both adaptive to the environment and more productive than local breeds (Aboagye, 2014; Ojango et al., 2017; Kebede et al., 2018). Daily milk production has on the average been reported to be about 1.9 L in an indigenous tropical cow and 5.9 L in crossbred cows with income level from milk yields of crossbred cows is 3.2 times higher than the indigenous cows (Quddus, 2017). Increase in milk yield also improves family nutrition and provides extra income to farmers (Manirakiza et al., 2017) thereby improving livelihoods. After many years of utilizing crossbreeding in this manner, it is important to review its impact on dairy cattle crossbreeding in the tropics. This will enable an objective assessment of its impact on milk production, dilution of local cattle genetic resources, available infrastructure and also indicate the role of stakeholders in helping to improve on dairy cattle production. The objective of this review therefore was to document the experiences, challenges, key lessons learnt and the best practices of smallholder dairy cattle crossbreeding as a tool for sustainable milk production and make appropriate recommendations to improve on future initiatives.

EXPERIENCES FROM DAIRY CATTLE CROSSBREEDING

Tropical dairy cattle are descendants of the Zebu cattle and well adapted to the tropics and tolerant to high temperatures and heat stress, partial resistance to diseases and pests such as ticks as well as their low nutritional requirements (Cunningham and Syrstad, 1987; Thornton, 2010; Aboagye, 2014; Osei-Amponsah et al., 2019). However, their genetic potential for high milk production is poorly developed (FAO, 2010) usually not letting down milk unless stimulated by sucking of the calf (FAO, 2013). On the other hand, exotic breeds of the temperate region have higher genetic potential for milk production. This provides the basis of crossbreeding to improve upon the tropical indigenous dairy cattle breeds for more milk yield. Crossbreeding combines the complimentary genetic and phenotypic characteristics of the adaptive potential of both *Bos indicus* of tropical regions and *Bos taurus* cattle breeds of temperate regions and increases heterozygosity and genetic variation (Aboagye, 2014). Many benefits such as increase in milk production, long lactation interval, and early calving age have all been some of the positive outcomes of crossbreeding. Furthermore, crossbreeding encourages involvement of NGOs in breeding programs to provide AI services, governments willingness in drafting policies that aids breeding programs and increase in diversity genetic resources of national population.

Consequently the low productivity of tropical cattle has made crossbreeding a viable option to improving their productivity to ensure profit (Aboagye, 2014; Roschinsky et al., 2015; Ojango et al., 2017). Crossbred cows are more economical and provide higher yield than the indigenous cows, and inclusion of a few crossbred cows can increase the income of a dairy entrepreneur and provide gainful employment of its family labour (Islam et al., 2008; Kebede et al., 2018). Consequently, crossbreeding has made many impacts on farmers and changed their management of pure-bred dairy cows. In many countries including India, crossbreeding of non-descript zebu cows with semen of exotic dairy cattle breeds has resulted in enhancing milk production by 5 to 8 times to that of non-descript cows, reducing age at first calving and shortening calving intervals in first generation crossbred progenies (Singh, 2016). Compared with milk production in the local cattle breeds of Ghana, milk yields increased dramatically in the F_1 crosses with Jersey and Friesian cattle with age at first calving and calving interval decreasing as the level of exotic breeding increased in the crossbreds (Aboagye, 2014). Galunkande et al. (2013) reported relative performance of indigenous breeds compared with different grades of crossbreds in terms of milk yield per lactation, age at first calving, services per conception, lifetime milk yield and total number of lactations completed. The findings indicated that at 50% *Bos taurus* blood, lactation milk yields were 2.6, 2.4 and 2.2 times higher than those of local cattle in the highland, tropical wet and dry, and semi-arid climatic zones, respectively; with superior lactation lengths and

Table 1. Reasons for success or failure of some cattle crossbreeding programs.

Crossbreeding type and breed of cattle	Country	Success	Reason
Rotational Crossing			
Holstein/Gir	Brazil	Yes	Increased profit
Synthetic Breed			
Mpawpaw	Tanzania	No	Lack of interest from local farmers
Breed Substitution			
Holstein	Kenya	Yes	Zero or semi zero-grazing systems; High potential agroecological area; Market linkage
Holstein	Ghana	No	Increased mortality due to low adaptation

Source: Leroy et al. (2016).

lower calving interval and age at first calving. The effect of indiscriminate crossbreeding in African countries are well known and sometimes visible as indicated in the gradual reduction of the population sizes of pure indigenous breeds with dilution of local adaptive germplasm (AUIBAR, 2019). Additionally, crossbreeding in most parts of the developing world has not always met the desired objectives. Table 1 gives an indication of the mixed results of cattle crossbreeding in selected countries.

CHALLENGES OF CROSSBREEDING

Despite the importance of dairy cattle crossbreeding, only few of the programs across Africa and other tropical countries have been successful. The major drawback in improving productivity especially in response to future demands for livestock products in sub-Saharan Africa is the absence of good infrastructure; absence of AI services; poor recording and monitoring systems (Roschinsky, 2013) as well as human and institutional resource constraints. Even though crossbreeding has great potentials and reliable outcomes which are observed in places where it has been in practice, it is not greatly adopted and applied in many parts of the tropics due to several challenges that may limit the advantages of heterosis and breed complementarity and jeopardize the sustainability of the system (Galukande et al., 2013; Roschinsky et al., 2015; Leroy et al., 2016). These include limited or non-involvement of farmers in the planning stages, shortfalls of crossbreeding methods, inadequate funding of crossbreeding programs, mismatches between genotypes and production environment and inappropriate recording and policy systems (Kebede et al., 2018). Limited involvement of farmers when designing crossbreeding programs, shortfalls of crossbreeding programs, inadequate funding of crossbreeding programs, environment and genotype mismatch, trained manpower problem were the major challenges facing smallholder dairy cattle crossbreeding in the tropics (Ojango et al., 2017). Additionally, genetic potential of crossbred cows is not fully exploited due to extreme climatic conditions and variable quantity and quality of feed resources in SSA (Roessler et al., 2019). Therefore, strategies designed to develop the dairy sector should take into account the existing production system and its unique characteristics of the area and should focus on a systematic approach to alleviate the identified constraints by involving all stakeholders in the formulation and implementation of improvement strategies (Moges, 2012; Ojango et al., 2017).

Many management (housing, health and nutrition) requirements of crossbred cattle are too complex and beyond the means of smallholder production systems (Galukande et al., 2013; Ojango et al., 2017). The smallholder sector in the tropics, which constitutes most of the farmers, is at times unable to raise the levels of management and nutrition in line with the requirements of the new genotypes due to resource constraints (Kahi, 2002; Kebede et al., 2018). Most tropical countries do not have adequate infrastructure and the cost involved, such as for transportation and the liquid nitrogen for storage of semen which has led to the failure in carrying out crossbreeding programs even though artificial insemination (AI) has been successful in the developed world (Hailu, 2013; Chebo and Alemayehu, 2012). The failure by smallholder farmers, who lack scientific understanding of crossbreeding, to recognize the unique needs of the different production systems has led to a low success rate of crossbreeding programs in many tropical countries (Galukande et al., 2013). Lack of policies in data collection, documentation and even maintenance of records has been found to be one of the threats to crossbreeding (FAO, 2007). The absence of technical skills and financial resources has been identified as the main obstacles in the establishment of sustainable animal recording systems in many African countries (FAO, 2007). Unlike the local breeds which can be fed through grazing where they walk many kilometres grazing, the crossbreds are kept under cut and carry feeding regime system of where they must be fed with supplements from industrial by-products and concentrates. This requires high labour requirements to feed, clean and manage intensively kept dairy cattle. These requires management skills and equipment such as feeding troughs, lighting system, shelter separation and disease management which are all additional tasks for smallholder farmers (Roschinsky et al., 2015). The crossbreds are also known to be more susceptible to diseases than purebreds and therefore a reliable and affordable veterinary service are required here to manage these diseases (FAO, 2007; Aboagye, 2014).

LESSONS LEARNT AND BEST PRACTICES

Demand for animal products is expected to increase due to human population growth, higher incomes, increased urbanization, and changes in dietary preferences resulting in a need for increased production (Osei-Amponsah et al., 2020). Crossbreeding has often been promoted with the assumption that crossbreds would produce greater outputs as a result of the contribution of the exotic breed to productivity and that of the local breed to environmental adaptation (AUIBAR, 2019). Institutional and human resource capacities, equipment, infrastructure, skilled labour, liquid nitrogen to store imported semen, heat detection, readily available insemination service and reliable means of transport are some of the prerequisites of a successful crossbreeding programme. Under favourable conditions, careful planning and long-term organization, crossbreeding schemes can be considered an effective strategy to reduce poverty among smallholders but it is not recommended for extensive resource-poor production systems. Developing countries often rely on crossbreeding to improve performance of livestock populations, usually because they have not been able to implement proper genetic evaluation and straight breeding programs in locally available breeds has not been considered feasible (Leroy et al., 2016). For instance, the importance of genotype-environment (G x E) interaction requires routine monitoring particularly because of increased global warming (Cheruiyot et al 2019). The failure to develop such breeding programs has been found to be related to poor infrastructure, insufficient capacity for management, lack of long-term commitment of research and investment, governmental and development institutes, and low involvement of smallholders in the implementation of the programs (FAO, 2015). Crossbreeding has been successfully introduced in some favourable areas in the tropics but not in all areas due to lack of long-term breeding strategies or the introduction of breeds that are not adapted to the tropical environments (Philipsson et al., 2011; Aboagye 2014; Kebede et al., 2018). Indiscriminate crossbreeding of indigenous breeds with exotic breeds without enough considerations of environmental conditions of production (Philipsson et al., 2011) should be discouraged. Significantly ex-situ conservation program of the local breeds has been proposed as an accompanying strategy to improve the sustainability of the crossbreeding programs (Manirakiza et al., 2017).

Lack of maintenance and promotion of breed standards and small population sizes limit the selection, multiplication and stabilization of crossbreds to form synthetic breeds (Hailu, 2013; Ojango et al., 2017). In addition, lack of analysis of the different socio-economic and cultural roles that livestock play in each situation usually leading to wrong breeding objectives and neglect for potentials of various indigenous breeds particularly in low input environments (Philipsson et al., 2011; Hailu, 2013) have all led to failure of crossbreeding in the tropics. For any crossbreeding program or initiative to be successfully implemented, the breeding environment or system should be greatly considered (Galukande et al., 2013; Kebede et al., 2018). There is need for better understanding of the genotype by environment interactions to match appropriate genotypes to the production systems (Roessler et al., 2019). Additionally, indiscriminate crossbreeding and breed substitution which are threats to indigenous livestock breeds and can lead to loss of ecologically important traits such as disease tolerance (AUIBAR, 2019) and should be avoided at all costs.

In designing a breeding program, the level of inheritance to be used in the crossbreeds needs to be considered with 50% exotic blood usually recommended (Galukande et al., 2013). Furthermore, stakeholder involvement in smallholder dairy cattle crossbreeding and production should be encouraged. These people are either government or private (non-governmental officials) who play various roles in ensuring a successful breeding program (Rewe et al., 2009) and include breeder’s organizations, institutions providing extension services, educational and training institutions, logistical and regulating functions involving credit, storage, transport and marketing facilities as well institutions providing incentives to increase production and productivity. In Brazil, for example, breeding programs for indigenous cattle have been successful because of the cooperation between breed societies, groups of breeders or private firms, universities and research institutions (Rewe et al., 2009). To bring about change in production practices that will lead to improved productivity of dairy systems within the countries, investments are needed: to improve measurement and documentation of animal performance; to build technical capacity at different levels to better design and manage genetic improvement; for research to improve the uptake of genetic technologies in key production systems; and in the infrastructure and processes that will deliver appropriate technologies to target populations (Ojango et al., 2017).

IMPLICATIONS AND RECOMMENDATIONS

Crossbreeding has led to higher milk production per animal, higher income for the families and provision of high-value food and is thus an important livestock improvement tool in the tropics especially where farmers can provide sufficient management for maintaining animals with higher input requirements and access to the milk market can be secured. This has happened in cases where stakeholders have carefully reviewed the production environment against the demands of crossbred cattle. Policies, market information and access, environmental conditions of the breeding community, characteristics of animal populations and infrastructure available should all be considered when designing crossbreeding programs. Another requirement for successful crossbreeding programmes is skilled human resource. In this regard training of smallholder farmers for instance in nutrition, heat detection, record keeping, AI and veterinary service delivery will be helpful. Additionally, the required infrastructure for improved management of crossbred cattle and access to market should be developed, training of stakeholders including small scale farmers with technical management and improved extension and veterinary service support to farmers. Finally, there is a need for more practical research to improve the implementation of sustainable long-term crossbreeding programs in developing countries. Sustainable use and conservation of local animal genetic resources, commitment of national governments, the farmers, meat and milk processors, retailers and consumers to support crossbreeding will be crucial in securing a robust and environmentally friendly milk industry in the tropics.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Rabbit production practices in Kiambu County, Kenya

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To document current rabbit management practices in Kiambu County, a survey using structured questionnaire was undertaken in 45 farms identified using snow ball sampling technique. Data collected were subjected to descriptive statistics. Findings revealed that the majority of the respondents (57.8%) kept more than 10 does while the most prevalent breed was New Zealand White (82.2%). The main purpose of keeping rabbits for the majority (54%) of the farmers was income generation. Rabbit keepers depended on locally available feed resources with the majority (82.2%) feeding a mixture of forages and concentrate. The majority of respondents (71.1%) weaned the kits at 8 weeks of age while does were rebred at 9 weeks after kindling on 68.9% of the farms. Treatment of sick rabbits was done majorly by the farmers themselves (60.5%). Constraints identified included high cost of feeds (88.9%), diseases (84.4%) and lack of markets for rabbits and rabbit products (71.1%). This study concluded that rabbit farming in Kiambu County is practiced on small scale characterized by limited resource allocation and small flock sizes which may not support a sustainable off-take rate to meet the intended purpose of income generation.

Key words: Rabbits, feed resources, health, Kenya.

INTRODUCTION

Over many years, domestic ruminants and chickens were the primary food animals among many Kenyan communities (Borter and Mwanza, 2011). However, due to decreasing landholdings and ever-increasing cost of cereal-based feeds, ruminants which need large quantities of forages and chickens which compete for cereals with humans there is need for alternatives. In view of this and with the necessity to increase food production for the growing human population, the need for a livestock species that can be raised easily and cheaply has emerged.

In the recent past, there has been an increased interest in rabbit farming in the country with emergence of many new rabbit farmers and the formation of distinctive self-help organizations such as Rabbit Breeders Association of Kenya (RABAK) based in Thika (Serem, 2014). Establishment of a rabbit abattoir in Thika town is also an indication of this increased interest. High prolificacy, rapid growth, lower input requirements, better feed usage and valuable output products (meat, pelt, manure and urine) are among valuable qualities of rabbits (Kale et al., 2016; Mutsami and Karl, 2020). In addition they can be produced from the enormous forages and feed materials that freely abound in the tropics (Chah et al., 2018).

With this upsurge in the production of rabbits, management practices currently utilized by farmers as it

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may impact on rabbit performance needs to be determined and farmers advised in order to make the venture profitable. This study aimed at documenting what farmers are doing with regards to rabbit breeding, feeding, and disease management in Kiambu county.

MATERIALS AND METHODS

The study was carried out in Kiambu county, which was purposefully selected on the basis of the population of rabbit keepers (MOLD, 2012), and the level of intensification and commercialization of rabbit production (Serem, 2014). The county occupies an area of 2543.42 km^2, and geographical coordinates of 1° 10' 0" South, 36° 50' 0" East. The county has a warm climate with temperatures ranging from 12 to 24°C with rainfall aggregate of 1000 mm annually. The main occupation of the majority of its inhabitants is crop and livestock production. Dairy cattle, poultry, pigs, sheep and goats are the common animal enterprises in the county. Although not traditional, rabbit keeping is gaining popularity in the county due to decreasing land size and increasing awareness of nutritional and economic benefits of rabbits as food animals.

Rabbit keepers in the county constituted the population for the study. Due to few numbers of rabbit farmers and the lack of comprehensive lists of rabbit farmers in the livestock offices a snow ball sampling technique was used to identify 45 rabbit keepers for the study. A cross-sectional survey was then carried out and data collected using a structured questionnaire which was pre-tested and then modified as necessary before being administered. On the basis of scheduled face-to-face interviews with rabbit farmers and personal observations, information regarding to feed types, sources, availability, presentation form, preservation, feeding times and constraints were collected from these farms. At the same time, data were collected on rabbit breeds as well as prevailing diseases, their treatment and prevention.

The responses were organized, categorized and coded to enhance analysis and entered into the computer. The data generated was subjected to descriptive statistics using Statistical Package for Social Science (SPSS) and results in form of percentages were presented using tables.

RESULTS AND DISCUSSION

Characteristics of rabbit keepers

The characteristics of rabbit keepers in the study area are shown in Table 1. Of the total keepers, 76% were males and 24% females. This gender disparity with a high predominance of males can be attributed to high prevalence of men as family heads (Osei et al., 2012) and cultural beliefs that rabbit farming is meant for men and boys (Borter and Mwanza, 2011). Over 93.3% of the rabbit owners were 30 years and above, an observation that negates the historical perception that rabbit farming is a pastime activity for young boys (Hungu, 2011; MOLD, 2012). Among the respondents, 24.4% were university graduates, 37.8% middle level college graduates while the remaining 20%, 13.3% and 4.4% had secondary, primary and no formal education respectively. This finding indicates that rabbit farming was not only being increasingly undertaken by the older persons but also by the educated. A positive relationship between farmer's level of education and productivity has been reported (Gasperini, 2000; Reimers and Klasen, 2013; Oduro-Ofor et al., 2014), this being attributed to the fact that farmers with higher education levels can think critically, make better decisions and choices (Oduro-Ofor et al., 2014) and more easily adopt new farming technologies (Mendoza et al., 2008), all of which have positive effects on productivity.

Most of the farmers (44.4%) owned 1 to 3 acres, 42.2% owned less than 1 acre while 13.3% more than 3 acres of land (Table 1). According to Serem (2014), land size was a significant factor in determining whether a household kept rabbits or not. Rabbit farming is gaining popularity among small landholders particularly those in urban and peri-urban areas mainly because they require less space than other types of livestock (Hungu, 2011; Mutsami and Karl, 2020). Majority of respondents (40%) had kept rabbits for <3 years while 36 and 24% had spent 3 to 7 and more than 7 years respectively. These results confirm that rabbit farming continues to attract new entrants in Kenya as reported by Kale et al (2016). According to Borter and Mwanza (2011), for rabbit production to gain hold in a new production systems, it takes on average 7 years given the right attitude and parent stock availability. More experienced rabbit producers would be expected to be better informed and able to improve farm productivity (Tembachako and Mrema, 2016). These researchers also reported that experience significantly affected commercialization of the enterprise and this was reflected in this study since the total average number of rabbits kept per respondent increased from 33.9±28.7 to 46±16.5 as years of rabbit farming increased from <7 to >7 years respectively. This increase in the number of rabbits kept with years of experience could also be due to a realization of the economic benefits of the enterprise as was observed by Sylvester et al. (2014) in Zimbabwe.

The main reasons given by rabbit keepers for the establishment of rabbit enterprises were income generation (54%), source of family food (33%) and both (13%) as shown in Table 1. These findings are in agreement with those of Serem (2014) in four rabbit producing counties in Kenya, that majority (51%) of the respondents kept rabbits for income generation. Other useful, but not primary reason for keeping rabbits were manure (91.1%), urine (51.1%) and pelts (8.9%). The main constraint to achieving the intended purposes of keeping rabbits for 71.1% of the respondents was a poorly developed market for rabbits and their products.

The main outlet for sale of rabbits was farmgate (75%) followed by local markets (21.9%) and hotels (9.4%). The mean age for an estimated 2 kg live weight rabbit was 4 months while the average cost per kg live weight was KES 350 (3.5US$). Lack of well-developed rabbit markets has been identified as a major constraint facing rabbit production in developing countries with farmers

Table 1. Characteristics of rabbit keepers in study area.

Variable	Category	Frequency (%)
Gender	Male	75.6
	Female	24.4
Age (years)	<30	6.7
	30-60	71.1
	>60	22.2
Education status	Graduate	24.4
	Certificate/diploma	37.8
	Secondary level	20
	Primary Level	13.3
	No formal education	4.4
Size of the land owned	<1 acre	42.2
	1-3 acres	44.4
	>3 acres	13.3
Years farmed rabbits	<3 years	40
	3-7 years	36
	>7 years	24
Reasons for keeping rabbits	Income generation	54
	Source of family food	33
	Source of income and food	13

Table 2. Breeds of rabbits kept by farmers in the study area.

Breed	Frequency	Percentage (%)
New Zealand white	37	82.2
California White	34	75.6
Crossbreed	32	71.6
Chinchilla	27	60
Dutch	25	55.6
Flemish Giant	18	40
Checkered Giant	13	28.9
Angora	4	8.9
French lopped	3	6.7
Palomino	3	6.7

who had previously kept rabbits citing lack of markets as the main reason for discontinuing the enterprise (Serem, 2014).

Breeds and breeding practices

The breeds of rabbits kept in the study area are shown in Table 2. Up to ten (10) rabbit breeds were kept with the three most commonly kept breeds being New Zealand White (82.2%), California white (75.6%) and crossbreeds (71.6%). Similar dominance of New Zealand and California White breeds has been reported by others in Kenya (Hungu, 2011; Serem, 2014) and their popularity associated with their good growth characteristics and high carcass weight. These two breeds are also widely accepted as meat breeds (Mailafia et al., 2010) and together with their crosses, were reported to attain a liveweight of 2 kg in 12 to 15 weeks under tropical conditions (Wanjala, 2015). The mean colony size was 37±19.1. Though the majority kept rabbits for sale and home consumption, the average number of 37 may be too small to sustain a family economically.

The main source of foundational stock was from other rabbit farmers (76.5%), a practice that denies farmers a guaranteed access to and continuous supply of quality and diverse breeds of rabbits including improved and/or imported ones (Oseni and Ajayi, 2008; Hungu, 2011) hence constrain productivity and quality (Mailu et al., 2013; Kale et al., 2016). Additionally, this practice can contribute to spread of diseases between farms (Ogolla et al., 2017). Replacement stock was mainly from own stock (89%) and exchange with other farmers (46.7%) with the later being done in order to prevent inbreeding. Serem (2014) dismissed the possibility that such methods would prevent inbreeding since most of the rabbits kept in the country originated from the government owned Ngong rabbit breeding centre. This could be further exacerbated by poor keeping of breeding records observed in the study area (28.9% of the respondents). Inbreeding leads to a general drop in performance of the animals due to reduced growth rates and increase in both mortality and frequency of hereditary defects (Kristensen and Sørensen, 2005; Nagy et al., 2012).

Breed (55.6%) and reproductive performance particularly litter size (37.8%) were the key determinants to breeding stock selection in the study area, in agreement with Hungu (2011). Breed choice is an important determinant of the productivity of a rabbit enterprise (Kumaresan et al., 2011; Fadare and Fatoba, 2018) since it has significant effect on mothering ability, body weight, growth rate among other traits (Sivakumar et al., 2013). Huish (2005) stated that consideration of rabbit performance while doing selection ensures passage of excellent traits on to the offspring which ultimately will improve performance. Reproductive performance, particularly litter size was the key determinant to breeding stock selection in the study area, in agreement with Hungu (2011).

The mean age at first service varied from 5 months (64.4%), 6 months (20%) to > 7 months (15.6%). Lebas et al. (1997) suggested that rabbits should be bred when they reach more than 80% of the their mature weight which is reportedly attained at 6 to 7 months of age depending on level of feeding (Serem 2014). The majority of kits (71.1%) were weaned at 8 weeks of age while does were rebred at 9 weeks after kindling in 68.9% of the farms. The average litter size at birth and weaning was 6 and 4 respectively and an estimated 90-day kindling interval. The weaning litter size of 4 was lower than 5 reported in other parts of the country by Serem (2014) but was within the range of 4 to 6 reported in Nigeria by Abu et al. (2008). However, the 90-day kindling interval was twice that recommended by the American rabbit breeders association of 45 days (ARBA, 2012). The combination of longer kindling interval with a small average weaning litter size would impact on the profitability of the rabbit enterprise as this translates to fewer animals for sale and/or slaughter for home consumption.

Feeds and feeding practices

At the time of the study, 65.8% of the keepers raised rabbits on a mixture of forages and concentrates, 25.4% on forages with or without concentrate depending on the availability, 13.3% forages alone and 4.5% concentrate alone. These findings are similar to those of Hungu (2011) and Serem (2014) who reported that majority of the farmers fed their rabbits on both forage and concentrates with the latter being fed only as and when one is able to afford them. According to FAO (2014), it is essential to ensure that animals receive adequate quantities of a balanced feed which is free from toxins and contaminants if productivity is to be maximized. A study by Wanjala (2015) showed that rabbits under forage-based feeding took five weeks longer to the target weight of 2 kg compared to concentrate-based feeding. Further, in the same study, the grower rabbits on the concentrate based diet had a better lifetime feed conversion efficiency mainly due to the reduced time to target weight.

Both fresh and dry forages were fed with Kales (66.7%), Rhodes grass (53.3%), Cabbage leaves (48.9%) and sweet potato vines (33.3%) being the most common. Other forages that were fed to the rabbits are presented in Table 3. The choice of forage offered to rabbits was based majorly on availability (57.8%) and this explains increased usage of Kales which is a common vegetable in most households. Other determinants were cost (35.6%), palatability (28.9%) and perceived nutritional value (13.3%) of the forage. Many studies on farm rabbit studies have shown that for most producers, the important determinant of feeds offered to rabbits is local availability (Aduku and Olukosi, 1990; Lukefahr, 1998; Chah et al., 2017). This may be indicative of the low level of commercialization of the rabbit enterprise as suggested by Serem (2014) in many areas of the tropics. Knowledge on quality of feed is important in determining how to best meet the quantities of nutrients required for maintenance and production.

All the keepers who fed forages to rabbits (95.5%) reported periodic forage scarcity mainly during the dry season. Despite the seasonality, only 29% conserved forages mainly as hay. Forages were also cultivated purposely for rabbits by 35.6% of the keepers; lucerne (25%), kales (56.3%), sweet potatoes (62.5%) and Napier grass (43.8%). According to Chah et al. (2017) very few farmers conserve forages for rabbits. This is an indication of lack of preparation for the dry season which may reflect a lack of information on feed conservation and/or a generally low availability of forages and therefore no surpluses to conserve (Serem, 2014). The low quantities required may also be the reason for lack of forage conservation by rabbit farmers.

High cost of rabbit concentrate feeds continues to make the use of forages in feeding rabbits a common practice especially in tropics as was noted by

Table 3. Forages used for feeding rabbits in study area.

Forage type	Percentage
Kales (*Brassica oleraceae var acephala*)	66.7
Rhodes grass hay (*Chloris gayana*)	53.3
Cabbages (*Brassica oleraceae var capitata*)	48.9
Sweet potato vines (*Ipomoea batatas*)	33.3
Kikuyu grass hay (*Pennisetum clandestinum*)	28.9
Gallant soldier (*Galinsoga parviflora*)	24.4
Household vegetable wastes	24.4
Napier grass (*Pennisetum purpureum*)	20
Black jack (*Bidens pilosa*)	15.6
Amaranthus (*Amaranthus spp.)*	13.3
Banana leaves and peels (*Musa spp*.)	11.1
Alfalfa (*Medicago sativa*)	8.9
Wandering Jew (*Commelina ensifolia*)	6.7
Maize leaves (*Zea mays*)	t4.4
Pumpkin leaves (*Cucurbitaceae*)	4.4

Iyegheerakpotobor and Muhammad (2008). However, the use of forages alone cannot sustain optimum productivity of rabbits thus the need for supplementation (Mailafia et al., 2010). This is especially so when such consist of tropical grasses, which are said to be relatively less digestible, low in protein and high in lignin compared to temperate ones (Mailafia et al., 2010). Rabbits raised on forage only have been reported to grow at a lower rate thus taking longer to attain slaughter weight (Wanjala, 2015). According to this author, rabbits on concentrate based diet grew at 202.5 g/week while those on forage diets 148.1g/week.

The majority of the farms (97.4%) purchased commercial concentrates while 2.6% use the home formulated. Chick mash was used in 10.3% of the farms. The amount of concentrate fed to rabbits ranged from 25 and 150 g/day per rabbit with an average of 91.5 g. Serem (2014) reported a considerably lower mean daily concentrate allowance at 70 g/day per rabbit. Majority of the farms (80%) did not keep proper records on rabbit feeding, thus, the amounts of concentrates offered to rabbits were estimated. Where forage constitutes the bulk of the rabbit diet, a minimum concentrate supplementation level of 25 g per day is recommended (MOLD, 2012). Supplementation is important to compensate for low nutritional value of available forage (Bösing et al., 2014).

Feed troughs for concentrate feeding were present in 88.9% of the farms while 28% had forage racks. The use of feeders has been reported to lead to better performance by rabbits (Brzozowski et al., 1998). This can be attributed to improved health and reduced mortality that may arise due to consuming feed contaminated with faeces and urine when rabbit feed is placed on the floor. Hanging of forages inside the rabbit cage at a comfortable height, as was the case in 35.6% of the farms, also helps prevent rabbits from trampling on and soiling the feed (Borter and Mwanza, 2011).

Eighty nine percent (89%) of the rabbit keepers provided rabbits with drinking water while 11% thought water was not essential to rabbits hence never provided it to the rabbits. Rabbits require water and will cease to eat if not provided with water (Tschudin et al., 2011). Bawa et al. (2006) reported a lower growth rate and an increasing mortality rate among water deprived rabbits and concluded that water should be availed to rabbits for at least 12 h per day for optimum performance. This, however, depends on environmental conditions primarily temperatures and relative humidity under which rabbits are kept as well as the moisture content of the feed.

Diseases and health management

Occurrence of different types of rabbit diseases/ conditions was reported in majority (84.4%) of the farms. The three most occurring conditions were bloat (68.9%), mange (62.2) and ear canker (42.2%) as presented in Table 4. It was noted that disease occurrence was common in farms keeping many rabbits (average 37.8 rabbits) compared to those keeping fewer (32.9). This high occurrence may be attributed to a higher level of intensification resulting in inadequate ventilation and sanitation of rabbit houses (Owen, 1976; The Mercks Veterinary Manual, 2010). In addition, increased intensification could lead to decreased management quality and increased confinement of animals (The Mercks Veterinary Manual, 2010) both of which have impact on disease occurrence. Rabbit health combined with feeding has a large effect on their production

Table 4. Common rabbit diseases and frequency in study area.

Disease/condition	% of farmers reporting disease	Most affected age group (%)		
		Young	Adults	All ages
Bloat	68.9	40	11.1	17.8
Mange	62.2	4.4	44.4	13.3
Ear canker	42.2	6.7	26.7	8.9
Pneumonia	37.8	20	6.7	11.1
Coccidiosis	26.7	-	6.7	20
Diarrhea	24.4	17.8	2.2	5.6
Snuffles	22.2	13.3	4.4	4.4
Eye infection	11.1	2.2	8.9	-
Worm infestation	4.4	-	-	4.4

performance (Martino and Luzi, 2008; Sanchez et al., 2012; Okumu et al., 2015).

Other studies have reported prevalent of such diseases in rabbit farms. Serem (2014) reported diarrhea, mange and bloat to be the most common while Hungu (2011) reported diarrhea and bloat. Aleri et al. (2012) investigating the occurrence of rabbits conditions in Nairobi, Kenya reported high incidences of ear canker, gastrointestinal conditions (such as bloat) and pneumonia. According to 46.7% of the respondents, feeding rabbits un-wilted forages was the main cause of bloat followed by abrupt change of diets (28.9%). In addition to the current noted causes of bloat, Ogolla et al. (2017) also reported use of excess pellets and poor quality feed.

These diseases can reduce production of rabbits to unprofitable levels. For instant, coccidiosis is a major cause of losses in rabbit production (Okumu et al., 2015) which impair their growth and utilization of feed (Soulsby, 2005). According to Elshahawy et al. (2016), mange which is caused by mites has become a common and major constraint in rabbit production. It causes rabbits to lose appetite, body condition and stunts the growth rate (Chah et al., 2018) leading to economic losses and animal welfare problems in rabbit farms (Sharun et al. 2019). It is highly contagious and can spread easily between sick and healthy rabbits (Chebet et al., 2018). In Kenya, coccidiosis and mange are ranked the most important diseases affecting rabbits (Okumu et al., 2015).

On occurrence of disease, majority (60%) self-treat the rabbits, called animal health providers (28.8%), slaughtered the sick ones (6.7%) or do nothing (4.4%). Self-treating of sick rabbits by the keepers is widely practiced among Kenyan farmers (Ogolla et al., 2017; Chebet et al., 2018). In the study area, this was attributed to poor accessibility of veterinary services/rabbit health experts (46.7%) and where accessible due to high cost (26.7%). Schiere (2004) reported that lack of both veterinary drugs and animal health experts impedes rabbit farming in the tropics. Of those treating their rabbits 46.7% used contemporary medicine, 20% traditional medicine while 33.3% combined both types of medicine. The knowledge on the management of rabbit diseases and drug use had been obtained either from non-professional sources (62.7%) including fellow farmers or professional sources (37.5%) including veterinarians. According Ashfaq et al. (2014), relying on traditional methods by farmers rather than seeking proper veterinary advice for livestock diseases treatment is detrimental to their incomes and development of the industry. Some of the keepers practiced some disease preventive measures including deworming (20%), coccidiostats in feeds (48.7%), isolation of sick and/or newly acquired animals (31.1%) and spraying of rabbit houses (8.9%).

Conclusions

This study concluded that rabbit keeping is still practiced on a small scale basis where the number of rabbits kept may not be able to generate enough income to sustain families. The major constraints to rabbit production are high cost and poor quality feeds, disease occurrence coupled with absence of rabbit specific drugs and health experts, and lack of markets for rabbits and rabbit products.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Urban dairy production and waste management in Oromia special zone around Finfine, Ethiopia

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The survey was conducted to assess urban dairy production and waste management system in Oromia Special Zone around Finfinnee, Ethiopia. The three study towns (Burayu, Sululta and Sebeta) were purposively selected due to the high potential for commercial dairy production. A total of 90 commercial dairy producers 30 from each town who at least own 10 dairy cows were randomly selected. The farmers interviewed individually using the survey questionnaire. The collected data were analyzed and the study revealed that 47.8 and 52.2% of the interviewed were female and male respectively. Next to daily laborers, household wives shared larger responsibility for feeding (21.1%), milking (28.9%) and cleaning (13.3%). The genetic composition of dairy cows in the study areas ranges from 50% exotic gene inheritances to pure (100%) exotic Holstein Friesian. Accordingly, 50, 62.5, ≥75% and pure Holstein Friesian cows account for about 24.4, 38.9, 24.4 and 11% of the herd, respectively. The major sources of feed were both formulated feed and feed that mixed at home (55.6%) and tap water (74. 4%). The average age at first calving, calving interval and days open was 2.26±.05years 20.8 ± 0.05 months and 161.76±34.80 days respectively. The major waste in the farm is manure (73.3%) and followed by feed left over (14.45%) and dust (12. 25%). High price feed, shortage of land, unavailability of dairy cow/heifer in time, feed quality, unavailability of feed in nearby area, diseases and lack of access to credit, shortage of water and inadequate training were among the major constraint of dairy production that need urgent intervention to utilize the untapped resources in the area.

Key words: Dairy cattle, urban, production, milk, waste.

INTRODUCTION

Urban livestock production constitutes an important subsector of the agricultural production system in Ethiopia. The contributions of urban livestock production to overall development include income and employment generation, poverty alleviation, and improvement of human nutrition and health (Azage, 2004). Keeping animals in urban areas is not new and nowadays the practice of keeping livestock in urban areas is increasing in many developing countries. Urban agriculture is generally characterized by closeness to markets, high

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competition for land, limited space, use of urban resources such as organic solid wastes and wastewater, low degree of farmer organization, mainly perishable products and high degree of specialization. By supplying perishable products such as vegetables, fresh milk and poultry products, urban agriculture to a large extent complements rural agriculture and increases the efficiency of national food systems (Veenhuizen, 2006). Throughout the developing world, and especially in Africa, animals are an important physical and financial capital for many urban households. In the African settings, broiler chicken, milk and eggs come from city farms or the suburbs (Moustier and Danso, 2006). As a regular source of income, they represent a form of savings. They may also generate additional physical capital in the form of manure (Prain, 2006).

However, the major problem in less developed countries is lack of recognition of urban agriculture as a major contributor to food self-sufficiency and its many other actual and potential benefits towards sustainable urban development. In most of these countries there is no even baseline data and information on the very activity of the industry. In Ethiopia, market-oriented urban and per-urban dairy production systems are emerging as important components of the milk production systems. These systems are contributing immensely towards filling in the large demand-supply gap for milk and milk products in urban centers where consumption of milk and milk products is remarkably high (Azage and Alemu, 1998). Unlike rural livestock production in the country, which has recently given great emphasis for development in order to fulfill the livelihood of smallholder livestock farmers, research and development interventions are limited for urban livestock production in general and dairy production in particular in Oromia special zone around Finfine. Therefore, taking into consideration of these gaps, this study is designed to assess urban dairy production system and waste management practices in Oromia special zone around Finfine, Ethiopia.

MATERIALS AND METHODS

Description of the study areas

The study was conducted in Oromia Special Zone surrounding Addis Ababa, Ethiopia. The Oromia Special Zone has seven administrative towns. It is situated at an altitude ranging between 1700-3600 masl. The average minimum and maximum annual temperatures are 23 and 36°C, respectively. With the bimodal rainfall patter, the mean annual rainfall is between 800-226 mm. The long and heavy rainfall is received during the months of June to September while the short and small shower is received during February to April.

Study population

Urban dairy farmers and dairy animals kept by the dairy farmers represented the study population. Sample size from the three urban towns was determined as indicated in the formula below. Cross-sectional and retrospective studies were conducted to collect data using questionnaire. On-farm observations and laboratory analysis were also conducted on relevant data.

Sample size determination

The sample sizes for data collection through dairy farmers' survey were determined by using the sample size determination formula proposed by Yemane (1967).

$$n=\frac{N}{1+N(e)^2}$$

Where, n= designates the sample size the researcher uses; N= designates total number of households heads. e= designates maximum variability or margin of error; 1= designates the probability of the event occurring. Accordingly, a total sample size was 90 urban dairy farmers/producers were selected from the three study sites. Then random sampling technique was applied to determine samples from each city and 30 dairy farmers were included from each urban town.

Sampling procedure

Two-stage sampling techniques were used to collect data. The first stage involved purposive selection of three towns out of the seven towns based on the practices and the availability of dairy farms in those areas. In the second stage, urban dairy farmers were selected randomly from the list of urban dairy farmers from each selected town. Key informant interview (KII) was also used for the purpose of data collection.

Data collection methods

The methods to be employed for collecting data in line with the objectives of the study involved questionnaire survey, key informants interview, on farm observations. Primary data were collected through interviews using a structured questionnaire.

Data analysis

All the collected data were coded and entered into a data-base using statistical package for social sciences (SPSS virgin 20). Descriptive statistics such as mean, standard error, percentiles, and frequencies were calculated.

RESULTS

Socio-economic characteristics

The socio-economic characteristics of the study sites are presented in Table 1. The study showed that 47.8% of the surveyed dairy farms were owned by female households while 52.2% of them were owned by male households. The average age of the dairy farmers was 41.5 years (ranging from 24 to 59 years). Larger percentages (96.7%) of the dairy farmers were married, 1.1% was single and 2.2% were widows. Most (51.1%) of the dairy farmers had no sideline business. Besides dairying, about 42.2% of the respondents run small

Table 1. Socio-economic characteristics of urban dairy farmers in the study sites.

Variable	Number of responds	Percentage
Sex		
Male	47	52.2
Female	43	47.8
Age (years)		
24-38	23	25.5
39-48	49	54.7
50-59	18	19.8
Average age	41.5	
Marital status		
Married	87	96.7
Single	1	1.1
Widows	2	2.2
Educational level		
No formal education	15	16.7
Primary education	39	43.3
Secondary education	23	25.6
Tertiary education	13	14.4
Farming experience (yrs.)		
0-3	18	20
4-5	23	25.6
>5years	49	54.4
Sideline business		
Small business	38	42.2
Civil servants	6	6.7
Dairy only	46	5.1
Family size		
1-3	10	11.1
4-7	67	74.4
8-11	13	14.4
Herd size		
10-15	74	82.2
16-24	10	11.1
>24	6	6.7

businesses (shopping and waving) while 6.7% were civil servants engaged in dairying as a sideline business. About 5.1% of the respondents exclusively engaged in dairying alone. The average family size was 5.5 persons (ranging from 1 to 11 persons per household). The average farming experience was 3.41 years, which ranged from 1 to 10 years. The herd size per farm ranged from 10 to 39 dairy animals with a mean of 13.77 animals per household. Highest number (43.3%) of the dairy farmers attended primary education (1-8), 25.6% attended secondary education (9-12), 14.4% attended tertiary education and 16.7% had no formal education.

Household labor allocation for dairy farm activities

Household labor allocation to dairy farming and related activities are shown in Table 2.

Table 2. Household labor allocation for dairy farm activities.

Activists	Husband		Wife		Daughter		Young son		Household maids	
	N	%	N	%	N	%	N	%	N	%
Feeding	14	15.6	18	21.1	1	1.1	-	-	56	62.2
Cleaning	13	14.4	12	13.3	2	2.2	2	2.2	61	67.8
Milking	9	10	26	28.9	4	4.4	-	-	51	56.7
Milk selling	20	22.2	48	53.3	10	11.1	-	-	12	13.3
Input purchasing	60	66.7	28	31.1	-	-	-	-	2	2.2

Table 3. Blood Level and parity.

Blood level dairy breed	Number of respondents	Percentage
50%	22	24.4
62.5%	35	38.9
>75%	22	24.4
More than 75%	11	12.2
Parity classes of dairy animals		
Heifers	30	33.3
After one parity	26	28.9
After 2nd parity	6	6.7
After 3rd parity	2	2.2
mixed all class	26	28.9

The study showed that all of the dairy farmers make use of both hired labor and family member labor for the farm operation. Major farm activities of the farms like feeding, cleaning, milking, milk selling and input purchasing and other related farming activities were run by household wives, husbands and daily laborers (hired labor). Most of the chores that require physical activities like feeding (62.2%), cleaning (67.7%), and milking (56.7%) were accomplished by hired labor. Next to daily laborers, household wives engaged in larger responsibility for feeding (21.1%), milking (28.9%) and cleaning (13.3%). But the wives were more responsible in milk selling (53.3%) and the husbands were more responsible in purchasing inputs (66.7%). Though daughters were involved in some less labor demanding dairy farm activities, the share of young sons was nonexistent except in cleaning, which accounts only 2.2% of the labor share. This provides compelling evidences that, unlike our forefathers, the present day cyber generations or youths are less interested in agricultural activities that demand continuous labor input.

Level of exotic gene inheritances and parity

The genotype of dairy cows in the study towns are indicated in Table 3. The genotype of dairy cows ranged from 50% Holstein gene inheritances to high-grade dairy cows that even exceed 75% exotic gene inheritances. In the study towns, dairy cows whose exotic gene inheritances range from 62.5 and above constitutes about 75.6% while dairy cows with 50% exotic gene inheritances accounts for only 24.4%. The majority of the farmers start up their dairy business with heifers (33.3%) followed by second parity cows (28.9%) and less interested in older cows (Table 3).

Feeds and feeding system

The types of feeds used by dairy farmers and feeding system are shown in Table 4. The study showed that 20% of dairy farmers purchased formulated feeds from commercial feed producers, 24.4% of the respondents purchased feed ingredients and mix at home while 55.6% of the respondent used both practices. The main source of formulated dairy feed was from commercial feed producers. Five private feed sources were commonly used by dairy farmers for dairy farms in the study area. It was observed that all home-mixed feeds were almost all prepared from local available feed ingredients like wheat bran, noug (Giyzotia abyssinica) seed cake, wheat middling, linseed cake, bean hulls, cottonseed meal, brewery by-product, teff straw and salt. Respondents

Table 4. Types of feed and feeding system.

Variable	Number of respondents	Percentage
Types of feed		
Formulated purchased	18	20
Mixed at home	22	24.4
Both formulated and mixed at home	50	55.6
Problems of purchased feed		
Low quality	8	8.9
Price of feed	54	60.0
Unavailability	28	31.1
Method of feeding		
Individual Feeding	62	68.9
Group feeding	28	31.1
Amount of feed provided /day/head		
Based on body weight	2	2.2
Based on milk production	48	53.3
Based on body weight and milk production	40	44.4
Provisions of green feed		
Yes	76	84.4
No	14	15.6

revealed that one of the major bottlenecks in the study area was high price for formulated feeds (60%), unavailability (31.1%) and low quality (8.9). The majority of the feeding system was individual (68.9%) followed by group feeding (31.1%). The amounts of feed provided per head per day were based on milk production (53.3%), body weight (2.2%) milk production (44.4%) and bodyweight only (2.2%). The study indicated that the majority of the dairy farmers supplement their cows with green feed (84.4%) while 15.6% of the respondents supplement their dairy cows with grass hay as basal diet.

Water sources and frequency of watering

Water sources and watering frequencies in the study areas are shown in Table 5. The study showed that dairy farmers in the study areas had three different water sources for their dairy animals. These include tap water, water hole and pond water. The majority 74.4% of the dairy farms in this study use tap water followed by water hole (14.4%) and pond water (11.1%). The majority of the respondents provide water to their dairy cows twice a day (64.44%) while 35.5% of the respondents provide ad libtum indicating that water was not a critical problem in the study sites. On the other hand, it can be concluded that dairy farmers in the study sites are well aware of the importance of frequent watering on the production performances and wellbeing of dairy cows.

Dairy cattle housing system

Housing condition and housing type of dairy cows in the study areas are indicated in Table 6. About 81.1% of the dairy farmers used the stanchion housing type, 17.8% used the loose type and 1.1% free stalls. All dairy farmers constructed a separate dairy cattle house from the main residence and constructed within the living fences of the dairy farmers. About 76.7% of the houses were not constructed according to recommended dairy housing design while 23.3% followed standard barn construction design. The present study revealed that 91.1% of the dairy houses have enough ventilation and light whereas the reaming 8.9% was not enough light and ventilation. According to the respondent, the main reason for not getting proper light and ventilation was due to the land scarcity inappropriate site selection. It was observed that most of the houses were not conducive for the rearing of dairy cattle based on dairy housing standards. They were poorly constructed in terms of housing orientation and ventilation.

In the study sites, respondents responded that 66.7% of the dairy houses had individual pen while 33% were managed in free stall. About 94.4% of respondents replied that dairy houses had maternity pen while 5.6% of the surveyed dairy farm did not have maternity pen. In the study area, 47.8% of dairy farmer practiced head to head cow standing followed by tail to tail (35.6%) while 16.7% of the respondents practice neither of the

Table 5. Water sources for dairy cows around Oromia special zone.

Source of Water	Number of respondents	Percentage
Hole water	13	14.4
Tap water	67	74.4
Pond water	10	11.1
Frequencies of provisions water		
Free accesses (*ad libtum)*	32	35.5
Morning and afternoon only	58	64.44

standards (free standing). The majority of the dairy house (83.3%) used only for dairy cattle and the rest (16.7%) mixed their dairy cows with other livestock like sheep. The floor design for housing was concrete (90%), stone-paved (4.4%) and ground (5.6%) types. About 97.8% of the roof in the farms from urban was rainproof (corrugated iron sheet cover). About 86.7% of the barns were with good drainage whereas, 7.8% had satisfactory drainage. The general farm hygiene condition of farms in the study site was generally satisfactory.

Disease and biosecurity measure

Disease management practices and dairy farm biosecurity practices are shown in Table 7. According to the respondents, 80.7% of the respondents call for veterinary services while 19.3% of them treat by themselves. All the respondents (100%) replied that their dairy cows received vaccination services from government service providers. The most commonly occurring diseases were swelling of the udder. Based on the information obtained from the town's veterinary officers, the most common bacterial diseases that occurred in the commercial dairy farms were mastitis and less frequently brucellosis. Among the viral disease, foot and mouth disease rarely occurred. The majority (78.1%) of the dairy farmers used dedicated boots and cloths like overall when they were entering into the dairy house as one of a biosecurity measure against diseases. It was observed that only 23.3% of the dairy farmers used foot dips at the entrance of the dairy houses. The common foot dips used by the dairy farmers were detergents like 'Berekina" which is not actually known for its effectiveness.

According to the dairy farmers, blind teat happed due to mastitis diseases mainly occurred on the farm that negatively affects milk quality and quantity production. About 97.8% of dairy farm had mastitis disease and only 2.2% reported no mastitis. Both external and internal parasite control could practice in the dairy farm 65.6 and 78.9% respectively while 34.4 and 21.2% of the respondents had no program to control external and internal parasite respectively. According to respondents 98.9% of the farmers were used annual vaccination, also all the dairy farmers were isolate the sick dairy cow from the health dairy cattle into isolation pen and 67.8% of dairy farmers cull their dairy cow when the dairy cow treated and no heal, old and low productivity while 32.2% of the farmers in the study area were not culled the animals until those cattle has died.

Institutional support and extension services

According to the present study, most of the dairy farmers (73.3%) received expert support from extension services while 26.7% had no experiences of receiving extension services. Institutional supports like training and veterinary services were provided by the urban agricultural offices while loan providers provide training on the financial management. It is interesting to note that about 35.6% of the respondents reported that they receive credit services to start dairy farming, which was not actually common in livestock investment. Almost a quarter of the dairy farmer (27.8%) received trainings before starting the business while about 25.5% of dairy farmers received training after they established dairy farms. The training they received ranges from a few days to one month. For instances, 8.9% of the respondents received the training for one month followed by a few weeks training (5.5%), few days (38.9%) while 46.7% of dairy farmers were had no training. The training was provided by various institutions like urban agricultural offices, micro and small-scale enterprises and NGO (religion organization) situated in the city. 52.2% of the dairy farmers get training by the urban agricultural office, and 1. 1% was by NGO.

Productive and reproductive performances

Average daily milk yield

The estimated average daily milk yield is presented in Table 8. The estimated overall mean daily milk yield based on the sampled household response and observation during the survey was 13.4±.4 kg/cow/day at the study site. A significant difference ($P<0.05$) of average daily milk yield was observed in different parity

Table 6. Dairy housing condition.

Variable	Number of respondents	Percentage
Type of house		
Free stall	1	1.1
Lose type	16	17.8
Stanchion	73	81.1
Housing condition		
With other livestock	15	16.7
Dairy cattle only	75	83.3
Standard or recommended house design		
Yes	21	23.3
No	69	76.7
Enough ventilation and light		
Yes	82	91.1
No	8	8.9
Provision individual pen		
Yes	60	66.7
No	30	33.3
Maternity pen		
Yes	5	5.6
No	85	94.4
Frequencies of house cleaning		
One time a day	23	25.6
Two times a day	60	66.7
Three times a day	5	5.6
As needed	2	2.2
Type of floor		
Concrete	81	90
Stone slab	4	4.4
Ground compact	5	5.6
Type of roof		
Rain proof	88	97.8
Not rain proof	2	2.2
Drainage		
Good	78	86.7
Satisfactory	7	7.8
Poor	3	3.3
Farm hygiene		
Good	74	82.2
Satisfactory	11	12.2
Poor	5	5.5

as well as the stage of lactation. The overall estimated mean yield at 1st parity, 2nd parity, and 3rd parity was 10.7±0.26, 13.33± 0.31 and 16.06±0.61, respectively. The overall mean of pick lactation was 18.13±0.39; this was highly significant ($p<0.05$) across average daily milk production. The mean yield of early lactation and late

Table 7. Productive and reproductive performance of animal.

Variable	Mean ±SE
Average daily milk yield (ADMY)/head/day	13.3±.4
Yield at1st Parity	10.7±.26
Yield at 2nd parity	13.33±.31
Yield at 3rd Parity	16.06±.61
Yield at early lactation	12.5±.35
Yield at pick lactation	18.13±.39
Yield at late Lactation	8.5±.23
Age at first parity	2.26±.05
Age at pick lactation	4.33±.08
Lactation animals in farm	8.27±.25
Length of lactation	7.52±.08
Days open	161.76±34.80
Calving interval(CI)	1.88±.05
Number of service per conception (NSC)	3.22±.58
Pregnant animals in farm	3.32±.182
Mastitis animals in farm	0.98±.11
Culled animas in farm	0.44±.11

Table 8. Common waste and methods of west removal.

Variable	Number	Percentage
Frequency of removing waste		
Every day	43	47.8
Two times a day	47	52.2
How to transport		
By wheel barrow	73	81.1
By polythene and hessian sack	17	18.9
Kinds of disposal methods		
Dispose in pit or damp site	56	62.2
Use as fertilizers in their own garden	27	30
Give free to the other farmers	7	7.8
Have you biogas		
Yes	4	4.4
No	86	95.6

lactation were 12.5±.35 and 8.5±.23 respectively.

According to the respondents, the average age at pick yield was 4.33±.08 year and the estimated lactation length mean in the study site was 7.52±.08 months. In the current study area, the overall mean of lactation animals, pregnant animals and culled animals on the farm were 8.27±.25, 3.32±.182, 0.98±.11 and 0.44±.11, respectively. The total average numbers of dairy cattle on the farm in the study site are 13.01±0.652. These are categorized in large urban commercial dairy farms.

Age at first calving (AFC)

According to the respondents of the study area, the average age at first calving was 2.26±.05 years. The age at first calving changes the heifer from a non-producing expensive item into an income-generating cow. Early AFC reduces unproductive periods and a higher the AFC will be the additional rearing cost of the animal (Panja and Taraphder, 2012). In the current study area even if the dairy cattle breeds are crossbreed, the age at first

Table 9. Major constraints of waste disposal in urban dairy farming.

Constraints	1^{st}	2^{nd}	3^{rd}	4^{th}	5^{th}	Index	Rank
Lack of convenient dump site	49	21	18	2	0	0.29	1
Nuisance from its odor	31	45	8	4	2	0.27	2
Lack of transportation	8	23	24	3	32	0.17	3
Lack of market for selling manure	0	0	20	55	15	0.14	4
Shortage of labor	2	1	20	26	41	0.12	5

calving was longer and its almost more than two years. Age at first calving is closely related to generation interval and, therefore, influences response to selection. In the current study area, heifers are usually mated when they are mature enough to withstand the stress of parturition and lactation.

Calving interval (CI)

Calving interval is a time elapsed between two consecutive successive parturitions. The overall estimated mean calving interval was 20.8 ± 0.05 months in this study area. Yifat et al. (2012) reported that crossbreeds have slightly shorter calving intervals than indigenous in Tatesa Cattle Breeding Center. According to the respondents, the CI in the study area is shorter than the local cattle.

Days open

Days open, the number of days between calving to conception, influences the profitability of the dairy industry. The overall estimated mean days open in the study site was 161.76±34.80 days. This current finding is agreed with (Zewdie et al., 2011; Belay et al., 2012; Hunduma,2012; Niraj et al., 2014) who recently reported the average length of days open of 85.6 to 197 days for crossbred dairy cows in Ethiopia.

Number of service per conception (NSC)

NSC is one of the measurements for reproductive efficiency. It expresses the fertility level of the dairy herds. The present study Data showed that the mean of the numbers of services per conception is 3.22±.58.

Dairy farm waste management practice

Common wastes in commercial urban dairy cattle farming

According to the urban dairy farmers, manure (73.3%) was the major waste in their farms followed by feed left over (14.45%) and dust (12.25%) depending on the type of housing system. The major constraints of waste disposal encountered by the urban dairy farmers of the study area were lack of convenient waste disposal pit, nuisance odor, and disinterest in daily laborers (Table 9). Furthermore, bulkiness is manure is inconvenient for transportation as facilities are lacking. According to the respondents, 52.2 and 47.8% of the farmers remove waste two times per day and one time in a day respectively. About 81.1% of the farmers were used wheel barrow for transport while 18.9% used polythene and hessian sack. About 62.5, 30 and 7.7% of farm wastes in the study area were disposed at the manure disposal pit, used it as a fertilizer in their own garden and given freely to neighborhood farmers, respectively. Animal manure is one of the best sources for producing biogas and generate energy for household consumption. Contrary to this fact, only 4.4% used manure to produce biogas and 85.6% of the respondents were not using the manure to produce biogas.

DISCUSSION

It is a reality that the proportion of male and female-headed dairy farms that run a dairy farm business varies from town to town and communities with different socio-economic and socio-cultural backgrounds. This study demonstrates that the cultural taboos that offer male-headed households a sole owner of dairy farms started changing and female-headed dairy farms are overtaking the business in urban settings. Relative comparisons show that the proportion of female-headed dairy farmers is relatively higher than the proportion reported by Belay et al. (2012) in Jimma Town (24%) but lower than reported by Assaminew (2014) who reported 60% female-headed dairy owners farm owners in Holeta Town. On the other hand, the proportion of female-headed dairy farms is comparable with the value reported (47.7%) by Haile et al. (2012) in Hawassa Town. In either case, it is interesting to observe cultural taboos that deny females to run dairy business are started shifting implying that the long-awaited concept of gender equality started bearing fruits in urban settings.

In any country, running of effective dairy business has a direct association with the level of literacy. In the present study, most of the interviewed dairy farmers were literate and attended formal education from primary to the tertiary level. The implication is twofold. On one hand, they run effective dairy business as they record every farm transaction related to health services, inputs and outputs. On the other hand, they can easily adopt full dairy packages, extension and advisory services given by experts. In fact, comparable results have been reported by previous studies (Fekede et al., 2013) who reported that 41% of the interviewed farmers attended primary education in the Addis Ababa milk shed. Therefore, it is unsurprising that the level of literacy of dairy farmers varies from town to town in the same country and from country to country depending up on the socio-economic and socio-cultural settings.

The current study revealed that most of the dairy farmers are within active and productive age group who can effectively run cumbersome dairy business. Results of the present study are similar with report of Berhanu (2012) who reported average age of 44.1 years and family size of 5.42 in the majority of urban settings in Ethiopia. Likewise, Azage et al. (2013) reported age range from 39.7 to 51.9 years in Mieso and Shashamane towns of Ethiopia, respectively. The average farming experience (3.4 years) in the present study was lower than the value (9.67 years) reported by Berhanu (2012) for the commercial urban dairy farms of Ethiopia.

The overall average number of crossbred cows owned per household in the study site is greater than the figure reported for medium farms (6.43 cows) in Bishoftu, Ethiopia (Mulisa et al., 2011). The variations could be attributed to differences in production objectives between urban to urban farmers, and also the lack of sufficient space to accommodate large herd size in urban centers.

The study revealed that all the dairy farmers used hired labor and family member labor for the farm operation. Household members and hired labor were participating in various dairy cattle management practices. This was dependent not only on the sex and age of the family members but on the type of activities (Kassu, 2016). Young son and daughter had a small involvement in all activity but next to hired worker's, household wife had higher involvement in the heavy part of the activity; this agrees with the work of Habtamu (2018). More women's contribution to the dairy labor force continued to be a heavy burden on women, in addition to their daily routines of preparing food and caring for the family. The higher participation of hired labor in this study agreed with those of other African counties, in urban dairy farms of Dar es Salaam, Tanzania (Kivaria et al., 2006), and Kisumu; in Kenya (Kagira and Kanyari, 2010) hired labor was used intensively in 97 and 76% of households, respectively. In relation to dairy breed, the result indicated that all urban dairy producers reared the crossbred dairy cows and this is in line with the results of Habtamu (2018). The present study result, agrees with the result reported by Staal et al. (2002) that dairy farming experience is positively related to the keeping of crossbred dairy cattle. Adoption of improved dairy cow technologies is expected to be negatively associated with the size of livestock ownership (Moll et al., 2007).

The study revealed that few of the dairy farmers of the study area used purchased formulated feed alone for feeding their cattle's and some of them used only feed that were mixed at home but the majority of urban dairy farm in the study area used both formulated feed and feed that were mixed at home. The current study result is not in line with those of Fekede et al. (2013); the urban and peri-urban dairy operations depend mainly on the natural pasture hay as a source of roughage feed in the central highlands of Ethiopia but the result complianced with those of Azage et al. (2013), agro-industrial by-products such as bran, middling, oil seed cakes and molasses are fed as supplement to crossbred dairy cows in urban and peri-urban areas. In the study area all of the dairy farmers used hay grass and straw as basal diet for their dairy cattle. Since there is the scarcity of formulated feed, the farmers mostly used feed that mixed at home. The common types of concentrates feed ingredients used included: wheat bran, noug seed cake, wheat middling, linseed cake, bean hulls and salt. Among the different ingredients used, noug seed cake and salt are the sole concentrates feed ingredients for home-mixed concentrate mixture in the study site. The present study agrees with those of Assiminew (2014) that the dairy farmers blend the concentrate mixture for crossbred dairy cows from wheat bran (42.60%), noug seed cake (34.20%), wheat middling (10.27%) and the remaining proportions from linseed cakes bean hulls and common salts in Holeta town.

The feeding methods of urban dairy farm in the study area were mostly individual feeding system and few of them were feeding in group and this is agrees with the study of Assaminew (2014) assessment of feed formulation and feeding practices for urban and periurban dairy cows around holetta, Ethiopia. More of the farmers in the study area determine the amounts of feed provided per head per day based on the level of milk yield only this is agrees with the work of Azage et al. (2013). Some of the farmers in the study area relied on the level of milk production and the dairy cattle life body weight but few of them were relied only on the life body weight of the dairy cattle rather than the level of milk yield; this may due to lack of experience for rearing dairy cow. The main source of water in this study site were tap water which is comparable to the report of Azage et al. (2013) who reported that the majority (71.8%) of the urban dairy farming system (Hawassa, Shashemene, Yirgalem,Dilla), in southern Ethiopia rely on tap water and Assimenew (2014) reported 76.4% of the Holeta town dairy production system. Regarding frequency of watering, most of the dairy farmers water their cattle twice a day in this study which is agrees with the report of Lemma et al. (2005) who reported that almost all the respondents watered their cattle twice in a day and few of farmers were provided water ad libtum. Actually watering frequency of dairy cattle depends on access to water sources, age structure of the herd, physiological stage of animals and season Azage et al. (2013).

The purpose of housing dairy cattle like other farm animals is to reduce climatic stress on the animals that hinder production, reproduction and proper growth and development (Yibrah et al., 2005). The highest stanchion housing type were observed in this study; these were higher than the finding of Mulisa et al. (2011) in Bushoftu town for the majority of medium dairy holders (71.4%). All roofs of the barn were rain proof which is higher than most of the farms in all scales of production; 78.8% kept their dairy cows under cow shed roofed with corrugated sheets of materials (Mulisa et al., 2011) in Bisheftu town. The general farm hygiene conditions in this study site were more hygienic than Asela town where 39% dairy farms were hygienic (Hunduma, 2013). All the dairy farmers constructed a separate dairy cattle house from the main residence and constructed within the living fences of the dairy farmers. The houses were not constructed according to recommended dairy housing design extensions package service; however, majority of them had enough ventilation and light. In the study, majority of the dairy house had individual pen and maternity pen. In this study, majority of dairy houses were used only for dairy cattle and few of them were mixed with other livestock.

The health of dairy stock was affected by inaccessibility of veterinary service, death, disease occurrence and expensive private veterinary service. There were similar reports of veterinary-related problem in Ethiopia and elsewhere. Provision of veterinary service in Ethiopia is inadequate and underdeveloped (Ayele et al., 2012; Jaleta et al., 2013). Kitaw et al. (2012) also reported that veterinary service was the least commercialized among inputs of dairying with provisions limited to drug vending. On the other hand, service from private veterinarians is expensive and with limited outreach. The most common sign frequently occurred in their farm were swelling of the udder and closure of the teat. The common bacterial diseases that occurred in the study area were mastitis disease and less frequency occurrence of brucellosis and also lameness was happed. According to the respondents whenever sick dairy cow were observed in their farm, major of the farmers called Vet doctor and few of them were treat by themselves. This result is similar with the result of (Habtamu 2018) mastitis, lameness and brucellosis diseases were affected dairy cows, which are diseases associated with intensification. In Kenya clinical mastitis (66.7 %), lameness (23%); Lumpy skin disease (23 %) were reported as major health problems (VanLeeuwen et al., 2012). This study revealed that the contact between extension serve agents and urban dairy producers was good and frequent. According to the respondents, most of the dairy farmers had access to extension services while few numbers them had no access to extension services. The present study agrees with those of Berhanu (2012) that about 60.4% of farmers did not access livestock extension services because of inadequate capacity of extension service and only about 16% of farmers received extension services such as veterinary and crossbred cows, milk value addition and market information. Institutional supports like training, extension and veterinary services were provided by the urban agricultural offices while credit services were provided by the micro finance of the urban office.

The estimated mean daily milk yield of 13.4±.4 kg/cow/day of this study is comparable to the report of Nigusu and Yoseph (2014) of 14.1 kg /day/cow in urban and secondary town dairy production systems in Adama milk shed. However, it is higher than the finding from Hawassa City of 10.32±1.5 kg/cow/day (Haile et al., 2012) and Dar es Salaam, Tanzania of 10.4±0.7 kg/cow/day (Gillah et al., 2013). There were significant different ($P<0.05$) the average daily milk yield was observed in different parity as well as the stage of lactation. The overall estimated mean yield at 1st parity, 2nd parity and 3rd parity was 10.7±0.26, 13.33± 0.31 and 16.06±0.61 respectively. The overall mean of pick lactation was 18.13±0.39; this was highly significant ($p<0.05$) across average daily milk production and also there was significant difference between the mean yield of early lactation and late lactation. The estimated lactation length mean in the study site was 7.52±.08 months. This value was shorter than those reported by Adebabay (2009) that reported result of 10.1 months in bure town. The present study result was comparable to the lactation length of dairy cows (7.29 months) at Meiso district (Kedija, 2008). The lactation length in crossbred cows observed in this study is slightly shorter than the lactation length of 11.7 months reported for crossbred cows in the Central Highlands of Ethiopia (Zelalem and Ledin, 2001). The average ages at first service (AFS) reported for cross breed heifers 2.26±.05 years in this study is similar with those of Kassu (2016) which reported 2.69±.08 years for crossbred heifers in Sidama zone.

The AFC obtained in the present study for urban dairy crossbred cows is shorter than the result reported by Asaminew (2007) that the average AFC was 37.6 months in Bahir Dar milk shed area. Average AFC obtained in the current study is shorter than the finding of Fisseha (2007) with the overall mean of AFC 43.13 ±1.7 months for Holstein Frisian cows in Alage. Age at first calving is closely related to generation interval and, therefore, influences response to selection. Early AFC reduces unproductive period and a higher the AFC will be the additional rearing cost of the animal (Panja and Taraphder, 2012). The reported average numbers of services per conception (NSC) of the study area were slightly similar with those of Adebabay (2009) that the number of service preconception cross bred cow in bure town was 3.91. The present result is greater than those of Tadesse and Tegegne (2018) and the number of services per conception reported around Mekelle, Bako research center; the overall least squares means in the Maksegnit town and Fogera cows were 2.1±0.1, 1.34±0.11 2.0±0.65 and 1.42±0.05, respectively. According to Mukassa-Mugerwa (1989), cows with values of NSC greater than two, are regarded as poor, the average natural service conception has 1.18 whereas artificial insemination users has 1.5 up to 2.3.

The overall estimated mean calving interval was 18.8±.05 months' in this study area which is comparable to the value reported by Gidey (2001) for Frisian x Fogera breed (18.6 months) and it was longer than those reported by Kiwuwa et al. (1983), Enyew et al. (2000); Obese et al. (2013). Niraj et al. (2014) reported about 459 days for crossbred cattle in Arsi region Ethiopia. The current calving interval value is shorter than the estimates of Mukassa-Mugrewa (1989) (25 months) in cross bred cattle. Long calving interval implies that farmer's income suffers because cows spend a greater portion of their lactation at low production levels (Swai et al., 2007).

The overall estimated mean days open in the study site was 161.76±34.80 days. This current study agrees with those of Zewdie et al. (2011); Belay et al. (2012); Hunduma (2012) and Niraj et al. (2014). The average length of days open recently reported for crossbred dairy cows in Ethiopia was 85.6 to 197 days. The average days' open value in this study was slightly shorter than for the report of Lemma and Kebede (2011) which is 176.8 days from Addis Ababa and 171.18 days of Alphonsus et al. (2014) from Nigeria. The reproductive performance of cattle, particularly the probability of conception, may be negatively associated with the magnitude and duration of negative energy balance in early lactation (Walsh et al.,2011).

Manure is among the most important contributions that livestock make to intensification and sustainability (Ehui, 2000). Adoption of improved manure handling techniques is crucial in stall fed cattle (Powell and Williams, 1995; Paul et al., 2009). The majority of manure in this study area was disposed in dump site and some of farmers were used as fertilizer in their very small garden farm. Limited numbers of the farmers were used biogas. The most common environment concern with animal wastes is that it affects the atmospheric air with offensive odors, release of large quantities of CO_2 and ammonia which might contribute to acid rain and the greenhouse effect. The most threats of urban dairy farms in this study area were displacement and complain from the neighbors improperly management wastes.

Recommendations

Based on the findings, the following recommendations could be drawn:

1) Provision of urban dairy husbandry training and extension services should be improved for the better efficiency of the commercial urban dairy cattle production.
2) Provision of credit facilities from financial institutions with lower interest rates can play a significant role to the expansion and improvement of the urban dairy cattle farming.
3) The high price of feed and its unavailability at required amount and quality is becoming a threat for the sector. Thus, it needs government and private investors' participation in the establishment of feed processing centres so as to provide a feed with a standard quality and a fair price which is an adequate.
4) Large number of AI service should be done wth less conception rate; this will increase day open cow and decrease production and reproduction. Thus, the research institute and educational organization should give training for AI technicians and fill their skill gap.

Provision of credit facilities from financial institutions with lower interest rates can play a significant role in the expansion and improvement of the urban dairy.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Assessment on artificial insemination service delivery system, challenges and opportunities of artificial insemination services in cattle production in Western zone of Tigray Region, Ethiopia

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The aim of the survey was to assess artificial insemination (AI) service delivery system and identify the challenges and opportunities of AI service in cattle production in Western Zone of Tigray Region, Ethiopia. Twenty Kebeles (lowest administrative units) and 353 respondents were purposively involved in face-to-face interview. Data analysis was performed using SPSS and mainly summarized by frequency and percentages. Chi-square test was the statistical method used to test proportions of variables. There was statistically significant (P<0.05) differences in AI service interruptions in both regular working hours and weekends and holiday hours. AI service interruption was a critical challenge in regular working days and in weekends and holidays. This is because 73% of the respondents reported that AI service was interrupted in Monday through Friday and 75% of the respondents also reported that Artificial Insemination Technicians (AITs) were not available to deliver AI service in weekends and holidays. Above half (63%) of the small scale farmers reported that AITs were very poor. Most of the very poor AITs were in midland (48%) and lowland (36%) agro-ecologies compared to the AITs in highland (16%) agro-ecology. Mobile AI service delivery system through motor bikes was the most common AI service delivery system used. The major challenges of AI service in order of their importance were lack of community awareness, poor animal management, unskilled AITs and inadequacy of AITs. The government and NGOs should solve the current challenges of AI service. The current approach and system of AI delivery should be restructured. Adequate numbers of skilled manpower should be assigned based on the standard numbers of breedable cows per AI center or per AIT. Moreover, agro-ecology based human management system and infrastructure development should be adequately addressed.

Key words: Communication means, AI service interruption, distance between homestead and AI service center, AI service delivery system, agro-ecology.

INTRODUCTION

The total cattle population of Ethiopia in the rural sedentary areas is estimated to be about 60.4 million. About 55% of the total cattle population constitutes female cattle whilst 45% of the total population are

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male cattle. 98% of the total cattle population are local breeds whilst crossbred and pure exotic breeds accounted for just about 1.5 and 0.2%, respectively (CSA, 2017/18). The most important factor for the success and profitable dairy and beef farming is efficient reproduction (Sheldon et al., 2006). Ethiopia began crossbreeding work in the early 1950s, however, the crossbreeding activities were not based on clearly defined breeding policy with regard to the level of exotic blood inheritance and the breed types to be used (Aynalem et al., 2011). The total number of crossbred female cattle produced through the crossbreeding programme for decades in the country is extremely low. This was due to the unsuccessful crossbreeding through artificial insemination (Sinishaw, 2004; Desalegn, 2008; CSA, 2011). In Ethiopia, crossbred dairy cattle are mainly crosses of Zebu and Holstein-Friesian (Nuraddis and Ahmed, 2017).

Profitability in livestock production is highly related to the reproductive success of livestock (Newton, 2014). The application of biotechnologies must include good practices in animal husbandry, animal health and nutrition, and reproduction (Bertolini and Bertolini, 2009). Artificial insemination (AI) is a valuable biotechnological tool, and most commonly used in Ethiopia over the last 30 years (Webb, 2003). AI has many advantages compared with natural service (Lima et al., 2010; Lamb and Mercadante, 2016). AI is a time dependent activity, and thus heat period could be passed away before the cows receive AI service during long journeys of cows for the service (Lemma, 2010). Practice of a good cow management and selection of cows which have good body condition score are the two most essential requirements for successful estrous synchronization and AI service (Getabalew and Alemneh, 2019). AI service in Ethiopia has not been successful to improve reproductive performance of dairy industry of the country (Desalegn et al., 2009).

Tesfay et al. (2019) reported on the assessment and analysis of the participatory agricultural production constraint appraisal of Western Zone of Tigray and noted that efficiency of AI service was poor. However, the causes and challenges for the poor efficiency of AI service in this region were not clearly known. Therefore, it was compulsory to assess AI service delivery system in cattle, and assess the associated risk factors and/or challenges and opportunities of AI service in the area.

MATERIALS AND METHODS

Description of the study area

The survey was carried out in Kafta Humera, Tsegede and Welkait districts. Kafta Humera district is the lowland part of Western Zone of Tigray Region, Ethiopia whereas Welkait and Tsegede districts are the highland areas of Western Zone of Tigray. Kafta Humera district has two agro-ecologies which consist of 86% lowland (*kola*) and 14% midland (weina dega). Welkait district also has two agro-ecologies which include 60% lowland (kola) and 40% midland (weina dega). Tsegede district has three agro-ecologies which comprise 70% lowland (kola), 22% midland (weina dega) and 9% high land (dega). Kafta Humera district is characterized by an altitude of 500 to 1849 m above sea level (masl), rainfall of 650-750 mm and temperature of 25-48°C. Welkait district is characterized by an altitude of 700 to 2354 masl, rainfall of 700 to 1800 mm and temperature of 18 to 25°C. Tsegede district is also characterized by an altitude of 680 to 3008 masl, rainfall of 1200 to 2500 mm and temperature of 12 to 35°C (Tesfay et al., 2019). Moreover, Kafta Humera district was covered by 33% of forestry land and 5% of pasture land/grazing land, Welkait district had 18% of grazing land and 19% of forest land whilst Tsegede district accounted 35% of forest land and 22% of grazing land (Tesfay et al., 2019).

Data collection and analysis

Sample size, sampling technique and data collection methods

The survey was conducted in twenty rural and peri-urban Kebeles (lowest administrative units), 353 small scale farmers and 10 artificial insemination technicians (AITs), and purposive sampling was used to select Kebeles, households of small scale farmers and AITs. Kebeles with more than ten AI beneficiaries and who bred animals in two and above breeding seasons were involved in face-to-face interview. A structured questionnaire was used to collect the data from each household.

Method of data analysis

SPSS software (SPSS, v20, 2012) was used to analyze the data. The data were summarized using descriptive statistics (frequency, percentage and mean) and index method. The index method was used in ranking the challenges and opportunities of AI service:

Index = sum of (3 x number of households who ranked first + 2 x number of households who ranked second + 1 x number of households who ranked third) given for each variable divided by sum of (3 x number of households who ranked first + 2 x number of households who ranked second + 1 x number of households who ranked third) for all variables.

Moreover, an asymptotic chi-square test (X^2-test) was computed to test significance of proportions.

RESULTS

Household characteristics

Most of the household heads (HHs) (93%) interviewed were males (Table 1). The mean household family size was 6.04±1.993. Majority (92%) of the interviewed HHs were married, and most (54%) of the interviewed HHs were in the age category of 46-65 years old followed by HHs (32%) in the age category of 36-45 years old. About 45% of the interviewed HHs attended lower primary school whereas 31% of the HHs interviewed were illiterate. Majority (80%) of the household occupation was livestock-crop mixed farming. Extensive production system (88%) was the production system followed by the households interviewed while very few (7%) of the households followed intensive production system (Table

Table 1. Household marital status, Household head (HH) gender and age, HH education level, household occupation and production system (N=353).

Marital status (N=353)	Frequency (%)	X^2	P value
Single	2(0.6)	847.782	0.000
Married	325(92.1)		
Divorced	12(3.4)		
Widowed	14(4.0)		
HH gender			
Male	328(92.9)		
Female	25(7.1)		
HH age (N=353)			
Age categories of HH			
Below 25 years	1(0.3)	354.408	0.000
25-35 years	21(5.9)		
36-45 years	114(32.3)		
46-65 years	189(53.5)		
Over 66 years	28(7.9)		
Educational level of HHs			
No education	108(30.6)	219.762	0.000
Lower primary school	160(45.3)		
Upper primary school	47(13.3)		
Secondary school	30(8.5)		
College	8(2.3)		
Household occupation			
Off farm and livestock production	28(7.9)	568.144	0.000
Livestock-crop mixed farming	281(79.6)		
Livestock production only	5(1.4)		
Off farm and mixed farming	39(11.0)		
Production system followed			
Extensive production system	312(88.4)	481.773	0.000
Semi-intensive production system	16(4.5)		
Intensive production system	25(7.1)		
Cross tabulation test			
Educational level * production system followed		19.059	0.015

Numbers in parentheses are the percent of respondents.

1). The relationship between educational levels of household heads and the production systems followed were negatively significantly (P≤0.05) different.

Availability of artificial insemination technicians (AITs) on regular working hours and in holidays and weekends, distance of homestead from AI service center, and means of communication used by the small scale farmers

Most (73%) of the respondents reported that there was interruption of AI service in regular working days of Monday through Friday. Moreover, about three fourth of the respondents also indicated that AITs were not available in weekends and holidays (Table 2). However, 90% of the AITs reported a contradictory opinion that as they provided AI service in weekends and holidays. When the AITs were not available, 28% of the respondents decided to pass the date without insemination and wait the next 21 day of estrus cycle whilst 47% of the respondents used natural service. All AITs reported that the main reasons for AI service interruption were lack of incentives and infrastructures.

Table 2. Availability of AITs Monday through Friday and in weekends and holidays (N=353).

Availability of AIT Monday up to Friday		X^2	P value
Categories	**Frequency (%)**		
Always available (regular service)	97(27.5)	71.618	0.000
Not always available (there was interruption)	256(72.5)		
Cross tabulation of agro-ecology (AE)*availability of AITs in Monday up to Friday			
Always available*AE			
Highland	26(26.8)		
Midland	66(68.0)		
Lowland	5(5.2)	39.523	0.000
Not always available*AE			
Highland	47(18.4)		
Midland	108(42.2)		
Lowland	101(39.5)		
Availability of AIT in weekends and holidays			
Yes, s/he was available	88(24.9)	88.751	0.000
No, s/he was not available	265(75.1)		
Cross tabulation of agro-ecology (AE)*availability of AITs in weekends and holidays			
Always available*AE			
Highland	22(25.0)		
Midland	61(69.3)		
Lowland	5(5.7)	33.735	0.000
Not always available*AE			
Highland	51(19.2)		
Midland	113(42.6)		
Lowland	101(38.1)		
Remedies taken by the farmers when AITs were not available			
Pass the date without insemination	99(28.0)		
Use natural mating	166(47.0)	30.295	0.000
AIT is available	88(24.9)		
Availability on weekends and holidays (AITs response)			
Available	9(90)		
Not available	1(10)		

Few respondents (6%) received AI service in a distance of 21-30 km whilst 32% of the small scale farmers obtained AI service travelling a distance of 11-20 km. However, 50% of the AITs reported that they travelled a distance of 11-20 km whereas 40% of them travelled a distance of 21 to 30 km (Table 3). The mean number of Kebeles per AIT was 4.2±1.3 and 60% of the AITs reported that the number of Kebeles were beyond their capacity. It was also noted that the mean number of cows and heifers inseminated per AIT/day in the breeding season was 6.7±8.8.

Payment per insemination on AI service and farmers` evaluation on the knowledge and skill of the AITs

Few respondents (27%) did not pay for the AI service (Table 4). Majority (63%) of the small scale farmers reported that the AITs were very poor whilst very few (6%) of the respondents rated the knowledge and skill of the AITs as very good (Figure 1).

Major challenges and opportunities in AI service

There were many challenges in AI service. Based on the index ranking, lack of community awareness was the prime challenge in AI service (Table 5). Poor animal management and unskilled AITs were the second and third challenges of AI service, respectively. Most (78%) of the respondents indicated that there were opportunities in AI service whilst 22% of the respondents reported that there were no opportunities. Milk market access and feed access were the first and second attractive opportunities

Table 3. Distance of homestead from AI service center and means of communication used by the small scale farmers (N=353).

Distance categories (farmers response)	Frequency (%)	X^2	P value
Household site (AIT come)	5(1.4)		
<1 kilometer (km)	12(3.4)		
1-5 km	115(32.6)	224.751	0.000
6-10 km	87(24.6)		
11-20 km	112(31.7)		
21-30 km	22(6.2)		
Cross tabulation of agro-ecology (AE)*distance between homestead and AI service center			
Household site (AIT come)			
Highland	4(80.0)		
Midland	1(20.0)		
Lowland	0		
<1 kilometer (km)			
Highland	3(25.0)		
Midland	9(75.0)		
Lowland	0		
1-5 km			
Highland	41(35.7)		
Midland	57(49.6)	121.510	0.000
Lowland	17(14.8)		
6-10 km			
Highland	25(28.7)		
Midland	38(43.7)		
Lowland	24(27.6)		
11-20 km			
Highland	0		
Midland	69(61.6)		
Lowland	43(38.4)		
21-30 km			
Highland	0		
Midland	0		
Lowland	22(100.0)		
Mean number of Kebeles covered by a unit of AIT	4.2±1.3	Minimum of 1	Maximum of 6
Did you think that you have Kebeles beyond your capacity?			
Yes		6(60)	
No		4(40)	
Distance cover (AITs response)			
1-5 km		1(10)	
11-20 km		5(50)	
21-30 km		4(40)	
Number of cows and heifers inseminated per AIT per day during the breeding season	6.7±8.8 (Minimum of 1 and maximum of 30)		
Means of communication (farmers response)		Percent (%)	
AIT visits us daily in the breeding season		12.9	
AIT comes to home when S/he wants		64.9	
We call AIT when we need him/her (mobile)		64.9	
We take our cows to the AI service center		13.7	

Table 4. Payment per insemination on AI service and farmers` evaluation on the knowledge and skill of the AITs (N=353).

Do you pay for AI service?	Frequency (%)	X^2	P value
Yes	258(73.1)	75.266	0.000
No	95(26.9)		
Cross tabulation of AE*payment for AI service			
Yes			
Highland	63(24.4)		
Midland	137(53.1)		
Lowland	58(22.5)	27.489	0.000
No			
Highland	10(10.5)		
Midland	37(38.9)		
Lowland	48(50.5)		
Amount paid per unit insemination (ETB)			
2.00	258(73.1)		
0.00	95(26.9)		
Farmers` evaluation levels on the knowledge and skill of AITs			
Very poor	221(62.6)		
Good	112(31.7)	172.085	0.000
Very good	20(5.7)		
Cross tabulation of AE* Farmers` evaluation levels on the knowledge and skill of AITs			
Very poor			
Highland	35(15.8)		
Midland	106(48.0)		
Lowland	80(36.2)		
Good		16.590	0.002
Highland	30(26.8)		
Midland	58(51.8)		
Lowland	24(21.4)		
Very good			
Highland	8(40.0)		
Midland	10(50.0)		
Lowland	2(10.0)		

ETB=Ethiopian Birr.

in AI service, respectively (Table 5).

DISCUSSION

Majority (93%) of the household heads (HHs) interviewed were males. The participation of female headed households in AI service was poor and future attention is needed. Most (54%) of the interviewed HHs were in the age category of 46-65 years old. 45% of the interviewed HHs attended lower primary school whereas 31% of the HHs interviewed were illiterate. The small scale farmers were academically poor and calls introduction of adult education because it is basic for the success of AI service. However, it should be noted that change in education level in Western Zone of Tigray, Ethiopia did not influence production system; educated farmers could not shift from extensive production system to intensive production system. Only 1.4% of the respondents were involved in sole livestock farming. The awareness of the community on sole livestock farming is poor and needs to demonstrate on sustainable livestock production and nutrition and food securities. Extensive production system (88%) was the prominent production system followed by

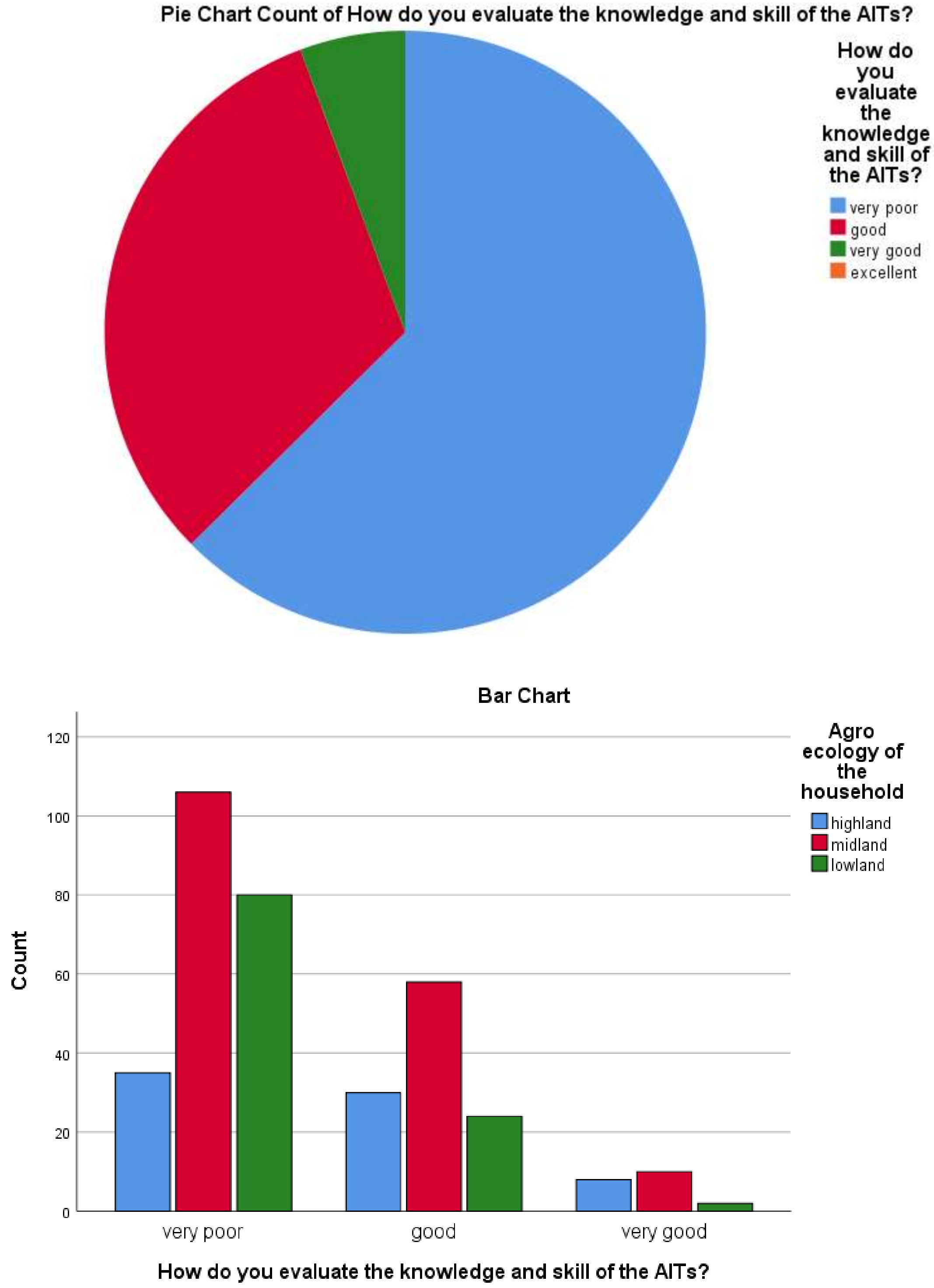

Figure 1. Evaluation levels of farmers on the knowledge and skill of AITs.

the households interviewed. The small scale farmers were resource poor and could not practice intensive production system.

The AI service interruption in Western Zone of Tigray, Ethiopia was a critical challenge because 73% of the respondents reported that there was service interruption in Monday through Friday and 75% of the respondents also reported that AI service was interrupted in weekends and holidays. A large proportion of AI service interruption was in midland (42%) and lowland (40%) agro-ecologies

Table 5. Challenges in AI service (N=353).

Challenges	R1	R2	R3	Index
Lack of awareness in the community	93	53	42	0.206
Delayed time of insemination	9	18	9	0.035
Inadequacy of AITs	49	61	36	0.147
Animal management problem	62	66	65	0.185
Heat detection problem	25	24	28	0.073
Unskilled AITs	76	38	29	0.160
Interruptions in holydays and weekends	19	44	62	0.100
Disease problem	5	7	19	0.023
Abortion	1	1	2	0.003
Dystocia	1	6	5	0.010
Lack of infrastructure	12	14	11	0.036
Long distance from AI service center	1	6	7	0.011
Poor communication	2	5	9	0.012
Opportunities in AI service				
Feed access	116	8	0	0.410
Market access (milk)	145	22	0	0.533
Best breed for crossing*	11	5	0	0.046
Presence of irrigation	2	2	0	0.010

R1=Rank one, R2=Rank two, R3=Rank three, *=Begait cattle.

of Western Zone of Tigray, Ethiopia as compared to highland (18%) agro-ecology in Monday through Friday. Almost a similar AI service interruption was reported in midland (43%) and lowland (38%) agro-ecologies as compared to highland (19%) agro-ecology in weekends and holidays. The variation in AI service interruption across agro-ecology could be due to the differences in weather conditions. However, 90% of the artificial insemination technicians (AITs) in Western Zone of Tigray, Ethiopia reported that they did not interrupt the AI service in weekends and holidays. The present AI service interruption in Western Zone of Tigray, Ethiopia is in line with the report 76% of respondents in Tiyo district and 60% of respondents in Sagure district did not receive regular AI service (Feyera and Tegenu, 2016); Ephrem (2019) reported lack of regular and consistent AI service (99%) and lack of weekend AI service (93%); Ashebir et al. (2016) reported that 60% of the respondents in Eastern and Southeastern of Tigray, Ethiopia did not receive AI service in weekends and holidays, 68% of the small scale farmers of selected districts of Arsi Zone did not receive AI service regularly, and 55% of the respondents reported that there was no service in weekends and holidays (Feyera and Tegenu, 2016), Riyad et al. (2017) reported that there was AI service interruption in weekends and holidays (51%) in Tullo district, West Hararghe, Ethiopia, 67% of AITs were not providing AI service in the weekends in West Gojjam Zone (Malede et al., 2013), Bemrew et al. (2015) reported that there was 66% AI service interruption in dairy cattle owners of Debretabour Town, Ethiopia Nuraddis et al. (2014) reported that 59% of the small scale farmers in Selected Districts of Jimma Zone, Ethiopia did not obtain regular AI service, and Tilahun and Yohanis (2018) reported 53% of the small scale farmers in and around Adama Town did not obtain regular AI service due to different constraints.

The AI service interruption in Western Zone of Tigray, Ethiopia is not in agreement with the report of Nuraddis et al. (2017) that 85% of the small scale farmers in Western Shoa Zone, Ethiopia obtained regular AI service. This large deviation of service could be due to differences in awareness of the small scale farmers and commitment of the AITs.

When there was AI service interruption, 47% of the respondents in Western Tigray, Ethiopia used natural service whilst 28% of the respondents reported that they decided to pass the cycle without insemination. A comparable 41% of the respondents in West Gojjam Zone used natural service whilst incomparable proportion of respondents (44%) in West Gojjam Zone decided to pass the cycle without breeding (Malede et al., 2013). Riyad et al. (2017) reported that 33% of the small scale farmers in West Haraghe, Ethiopia used natural service during AI service interruption. When the AITs were not available to deliver AI service, 28% of the small scale farmers in Western Zone of Tigray, Ethiopia decided to pass the date without insemination. This is in agreement with the report of Bemrew et al. (2015) in dairy cattle owners of Debretabour Town, Ethiopia because 29% of the small scale farmers decided to pass the date without insemination. But the proportion of small scale farmers

(47%) in Western Zone of Tigray, Ethiopia who used natural service and the proportion of the dairy cattle owners (28%) of Debretabour Town, Ethiopia who used natural service are different. Moreover, 62.5% of the small scale farmers in Selected Districts of Jimma Zone, Ethiopia decided to pass the estrus cycle without insemination (Nuraddis et al., 2014) when AITs were not available to deliver AI service. These differences in decision on whether to use natural service or pass the cycle without insemination may be due to differences in awareness of the communities and on availability of bulls for natural service However, the present AI service interruption in Western Zone of Tigray, Ethiopia is not in line with the works of Riyad et al. (2017) who reported that 44% of the respondents in West Hararghe did not use regular AI service whilst 56% of the dairy cattle owners obtained AI service without interruption. Ashebir et al. (2016) reported that 25% of the small scale farmers in Eastern and Southeastern of Tigray, Ethiopia did not obtain regular AI service. These differences may arise from the differences in awareness of the communities, distance between homestead and AI service center and commitment of the AITs.

The distance between homestead of small scale farmers and AI service centers of Western Zone of Tigray, Ethiopia was largest in midland and lowland agro-ecologies compared to the distance in highland agro-ecology. Some of the small scale farmers (14%) in Western Zone of Tigray, Ethiopia took their cows and heifers to the AI service centers and is not comparable with the study of Feyera and Tegenu (2016) which reported 61% of the small scale farmers took their cows to the AI service station whilst the remaining used AIT call to their vicinity. This difference may arise from differences in community awareness on AI service. Small scale farmers in West Gojjam Zone trek their cows for more than 28 km to AI service center due to lack of transport facilities by the AITs (Malede et al., 2013) is not in line with animal trekking distance in Western Zone of Tigray, Ethiopia because most of the animals trekked 1-20 Km to the AI service center(s).

A daily mean of 6.7±8.8 of cows and heifers were inseminated in each breeding season in Western Zone of Tigray, Ethiopia. Riyad et al. (2017) reported that average daily numbers of cows inseminated by AITs ranged from 1 to 10. One local AI center for every 5,000 breedable cows is a rule of thumb in AI service (Raymond and Saifullizam, 2010). Above half (63%) of the small scale farmers in Western Zone of Tigray, Ethiopia reported that AITs were very poor in their knowledge and skill. It was also noted that a large proportion of the small scale farmers who reported the existence of very poor AITs were in midland (48%) and lowland (36%) agro-ecologies compared to highland (16%) agro-ecology. This variation may be from the differences in academic background of the AITs and the level of provision of on-job trainings. The technical knowhow of most AITs in Western Zone of Tigray, Ethiopia was under the classification of very poor (63%) and is not in agreement with Desalegn (2008) report on the evaluation of the technical knowhow of AITs that they were categorized as good (57%) and very good (27%), poor (10%) and excellent (10%), and Malede et al. (2013) report on AITs in West Gojjam Zone were categorized as excellent (15%), very good (40%), good (23%) and poor (22%). This difference may arise from academic background of the AITs and on the frequency of access to on-job trainings.

The main challenges of AI service which negatively influenced the efficiency of AI service in Western Zone of Tigray, Ethiopia were lack of awareness in the community, animal management problem, unskilled artificial insemination technicians (AITs) and inadequacy of AITs. The present challenges are similar with Ashebir et al. (2016) reported that lack of awareness (18%) and management problem (14%) were some of the major constraints of AI service in Eastern and Southeastern Zone of Tigray. Bemrew et al. (2015) reported inadequacy of artificial insemination technicians (AITs) (16%), one of the major problems identified in Debretabour Town, Ethiopia, Malede et al. (2013) reported that inadequacy (60%) of AIT (Sekela district of West Gojjam Zone), distance to AI service center and unskilled AITs were the main problems in AI service in West Gojjam Zone. Ephrem (2019) reported that lack of regular and consistent AI service (99%), lack of weekend AI service (93%), inadequacy of AITs (92%) and lack of awareness (92%) were the major constraints in AI service in selected Districts of Wolaita Zone, Ethiopia. Riyad et al. (2017) reported that shortage of AITs (18%) and discontinuation of service on weekends and holidays (51%) was one of the problems in AI service. Tehetna et al. (2015) reported that lack of awareness (22%) and lack of AITs (18%) were among the major constraints of AI service in and around Alamata District, Tigray, Ethiopia, Belete et al. (2018) reported animal management problems (19%) and lack of skill in AITs (6%), and all are similar with the current challenges in Western Zone of Tigray, Ethiopia. These all reports indicate the limited number and skill of AIT are national challenges, therefore, the government should primarily solve the limited number and skill of the AITs.

All the major constraints in AI service in smallholder dairy cows of Harar were fed shortage (93%), heat detection problem (82%) and AI service charge (72%) (Engidawork, 2018) are not similar with the constraints in Western Zone of Tigray, Ethiopia. This is because all agro-ecologies and production systems could not have similar challenges; the challenges depend on the existing conditions.

CONCLUSION AND RECOMMENDATION

Education level of the households interviewed was very poor because 31% of the households were illiterate whilst

45% of the households attended lower primary school. There was poor AI service delivery system and poor AI coverage in Western Zone of Tigray, Ethiopia because there was little emphasis on AI service. Lack of community awareness, poor animal management, unskilled Artificial Insemination Technicians (AITs), AI service interruption and inadequacy of AITs were the main challenges in AI service. Therefore, many stakeholders should be involved in AI service to solve the existing challenges of AI service. The participation of female headed households in AI service was extremely poor. There were some opportunities in AI service, however, there was no effort to use the opportunities.

The mean and maximum number of Kebeles covered by a unit of AIT was four and six Kebeles, respectively. AI should not be practiced in scattered communities approach because AI service in scattered communities is not achievable. Therefore, AI should be practiced based on community-based approach to allocate adequate resources and particularly adequate number of skilled manpower should be assigned to each community. AITs should be provided frequent on-job trainings on how to detect heat and on effective insemination procedure, and they should be provided achievement-based incentives to enhance overall motivation and solve interruptions because the main reasons for AI service interruption were lack of incentives and infrastructures. The consequences of AI service interruption were that the farmers were exposed to uncontrolled natural service (47%) and pass the cycle without insemination (28%). Therefore, special commitment should be built in each AIT and institutionalized all day hours AI service system should be set. The community should also be provided adequate training on how to detect heat of cows and on proper husbandry practices. The types of communication channels were very limited (cellphone based), and many types of communication channels (magazine, newspaper, television, radio, and social media) should be used to create awareness in the community for the ease and sustainable use of AI service. There was negative relationship between educational level of household heads and production systems they followed. Educated households in the area did not practice intensive cattle production system. Household occupation system and livestock production system were basic constraints in AI delivery system because 80% of the households were involved in livestock-crop production system and 88% of the households followed extensive animal production system. Extensive cattle production system negatively affected AI delivery system. Therefore, AI delivery system is also successful when household occupation system is only involved in cattle production and when improved cattle management is practiced.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Native chicken farming: A tool for wealth creation and food security in Benin

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Poultry is the second largest livestock in Benin (contributing to 22% of the total meat produced), and chickens are the most reared poultry in the country. The objective of this study was to assess the current trends of local chicken breeding and factors that influence their production performance, and thereby to deliver information for production improvement and sustainable use of indigenous chicken breeds. An electronic tool was used to collect information from 269 respondents selected through a chain referral sampling method. The results showed that there were three production systems; however, the most common production system used was free-range extensive system (70%). Regarding flock ownership, men owned the birds in the majority of the cases (63%) and had overall higher population of chickens than women. The most predominant constraints for chicken production, in descending order of importance, included theft, access to bank loan, lack of training, limited investment, disease spread and mortality. From our results, gender, financial resources, main activity of the flock owner, weaning practice, and the production area had a significant effect on flock size. To enhance the indigenous chicken production, changes in traditional management practices combined with breeding program focusing on within breed selection (closed nuclear system) is a better approach.

Key words: Biodiversity, cluster analysis, food security, farmer livelihood, local chickens.

INTRODUCTION

Benin faces the challenge of increasing food production and reducing poverty. One option is to intensify agricultural production and diversify into more profitable and competitive livestock enterprises. According to a previous study, managing livestock in Africa is likely to be more profitable than growing crops under future climatic conditions (Dinar et al*.,* 2012). In Benin, the livestock subsector contributes approximately 6% of the agricultural Gross Domestic Product (USDA, 2014). Poultry is the second most important livestock enterprise in rural households where more than 70% of the country's population live and derive their livelihood (FAO,

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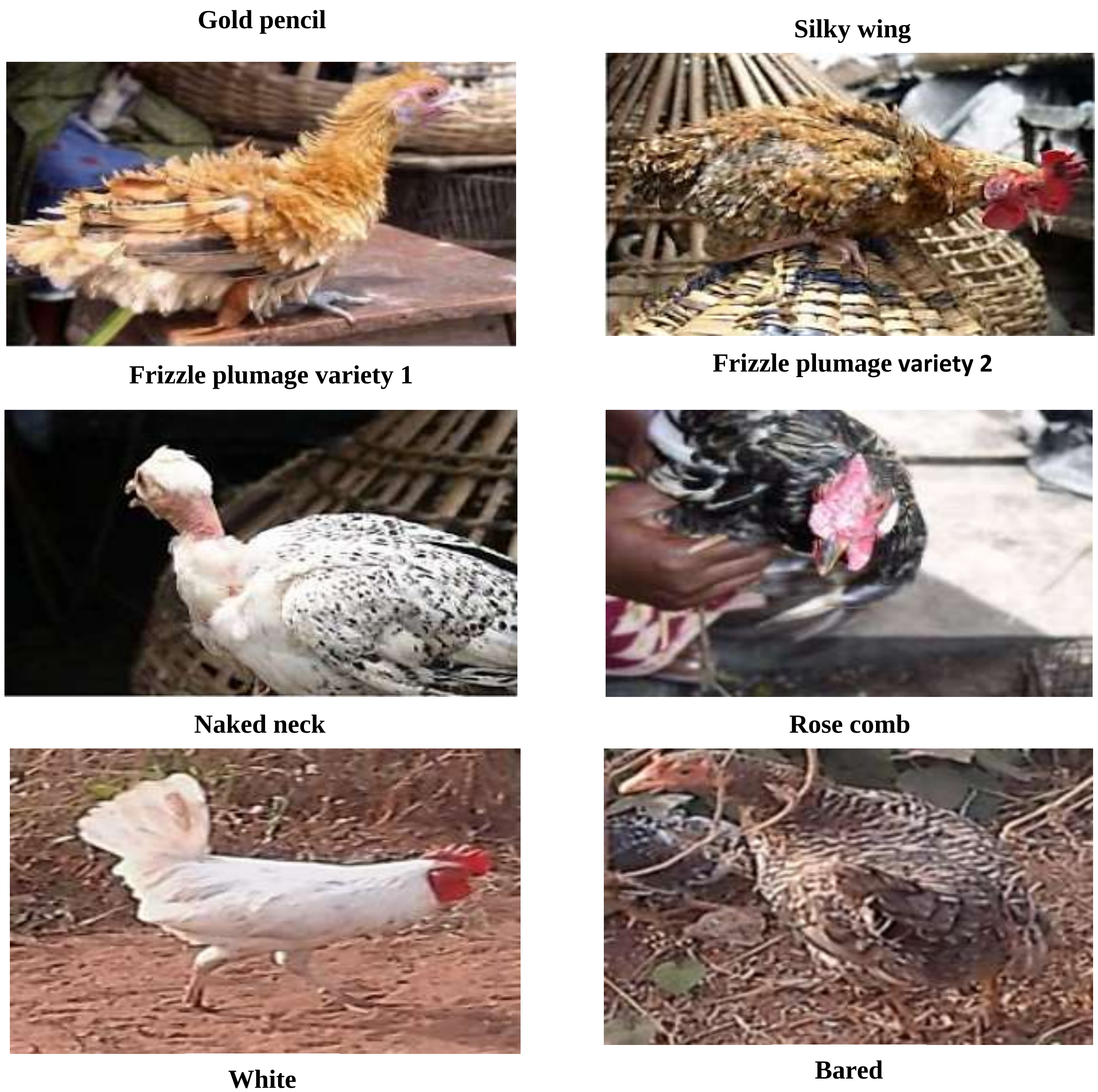


Figure 1. Documented breeds of local chickens.

2015). It contributes up to 22% of total meat production and the major poultry species kept are chickens, guinea fowls, ducks, turkeys, and pigeons with chickens dominating (Fanou, 2006). Therefore, chickens form an important component of livelihoods as a source of protein food security, income, insurance against emergencies, and have the potential for commercialization and wealth creation. The chicken of importance in Benin is the indigenous chicken (*Gallus domesticus*), with an estimated population of about 19,830,000 in 2017 while hybrid chicken number was estimated at 8,13,000 (DE, 2018). The diversity in agro-ecology, climatic conditions, and variation in the purpose of chicken rearing in different regions and production environments in the tropics are believed to contribute to the current high diversity in chicken genetic resources (Padhi, 2016). In Benin, there are approximately eight different breeds of local chickens that have been documented (Figure 1) (Tchabi, 2008). Although local chickens have a great potential for development, smallholder farmers keeping them face the challenge of improving the productivity of their flock for increased food products and income (Tchabi, 2008). Major constraints include socio-economic factors, erratic and unpredictable weather, zoonotic diseases and pests, inadequate capacity for service delivery, weak extension services, and demographic factors among others. Benin's poultry meat demand was estimated at 40,000 MT in 2018 while the domestic poultry meat production was just about 10,000 MT. However, a conservative estimate of poultry meat imports into Benin that year exceeded 2,00,000 MT (Aguehounde 2018; Dognon, 2018). This implies that poultry meat imports into Benin not only cover national poultry meat deficit but also Nigeria and hinterland country's needs.

The purpose of this study is to provide information about the current trends and the key factors influencing local chicken production in tree major chicken farming regions. The study then would give recommendations to the relevant authorities and the indigenous chicken farmers to address those factors aimed at increasing indigenous chicken production and subsequently improving the standard of living of the community through poverty reduction while maintaining the genetic attribute of the indigenous chicken that is appreciated by the consumers and producers.

MATERIALS AND METHODS

Sampling procedure and Study area

This study was conducted from July to October 2019 using the animal genetic resources characterization, inventory, and monitoring tool (AnGR CIM Tool) across, peri-urban and rural areas in 3 regions including Oueme, Zou, and Colline. The AnGR CIM Tool is a comprehensive tool designed by the African Union Inter-African Bureau for Animal Resources (AU-IBAR) using the Open data kit (ODK) software version 1.22.4 (AAGRIS, 2019). The tool was installed on a tablet with the latest version of Android OS. Due to resources limitation, municipalities where local chicken breeding is the most popular within each region were considered. These include Misserete and Adjarra in Oueme; Abomey, Bohicon, Za-kpota and Zogbodomey in Zou; Dassa and Glazoue in Colline (Figure 2). A total of 269 respondents (92 in Colline, 91 in Oueme and 86 in Zou regions, respectively) were selected using a chain referral sampling method. In order to ensure sample diversity, different sample seeds (initial subject) were identified as suggested by Kirchherr and Charles (2018). The study population within department is considered to be homogenous as communities living in the selected areas are close to each other and have similar livestock keeping practices. Therefore, going by Singleton et al. (1993), the sample size within each region would be considered adequate.

The Oueme region is subdivided into nine municipalities located in southern Benin in a coastal area that has interconnected lakes and lagoons and elongated coastlines with wide marshes. It receives two spells of rain from March to July and September to November, with an average annual rainfall of less than 1,200 mm (McColl, 2014). According to Benin's 2013 census, the total population of the region was 11,00,404, with 5,34,814 males and 5,65,590 females (Census of Benin, 2013). Oueme's indigenous chicken population was estimated at 9,44,766 in 2015 (PAFILAV, 2015).

The region of Zou is also located in southern Benin and subdivided into nine municipalities. It receives two spells of rain from March to July and September to November (McColl, 2014). In 2013, the total population of the region was 8,51,580, with 4,07,030 males and 4,44,550 females (Census of Benin, 2013). Zou's indigenous chicken population was estimated at 12,13,918 in 2015 (PAFILAV, 2015).

Collines is located in central Benin and is subdivided into six communes (DESA, 2004). As of 2013, the total population of the region was 7,17,477, with 3,53,592 males and 3,63,885 females (Census of Benin, 2013). It receives one season of rainfall from May to September (McColl, 2014). Colline's indigenous chicken population was estimated at 14,42,911 in 2015 (PAFILAV, 2015).

Data analysis

The data collected were downloaded from the AnGR CIM Tool platform and analysed with the version 3.5.1 of the R software (Team, 2013). Research methodology utilized both descriptive and inferential analysis. Descriptive statistics was used to establish the general characteristics of the study sample analysed that were compared using the bilateral Z test in Agricola Package in R software. For each relative frequency P, a margin of error (ME) was calculated using the formula: $ME = 1.96\sqrt{\frac{(P(1-p))}{n}}$ where p is the relative frequency and n the sample size (Lesaffre, 2009). Under inferential analysis, the Tukey test was used on one-way analysis of variance to determine the influence of independent variables on the flock size. Also, a Gower distance analysis was performed to cluster the mixed data using the 'gower_dist' function in the 'gower' package ver. 0.1.2 in R software (van der Loo, 2017). A descriptive analysis was performed on the clusters based on selected variables and differences between clusters were tested through chi-square test (frequencies) and T-student test (mean) where necessary.

RESULTS

Demographic factors that influence indigenous chicken production

Demographic factors likely to influence local chicken production are presented in percentages and frequencies in Table 1 and Figure 3. These include gender, education levels, and age of the respondents.

Table 1 shows that in general most of the chicken farmers (63%) were males. Comparison within regions yields the same result. Inclusively in the three regions of the study, male respondent rate was significantly higher than female, that is, 68, 62 and 59%, respectively in Colline, Oueme and Zou. The general illiteracy rate among respondents was 27%; however comparison within region exhibits different pattern. This rate is very low in the region of Zou (6%) and very high in the region of Oueme (43%). The information in Figure 3 shows that majority of chicken farmers belong to the age category of 41-61 years. However, chicken farmers in the region of Colline were much younger as majority of them were between 21 to 41 years old (42%).

Socio-economic factors that influence indigenous chicken production

Socio-economic factors likely to influence local chicken production include the average number of chickens in the

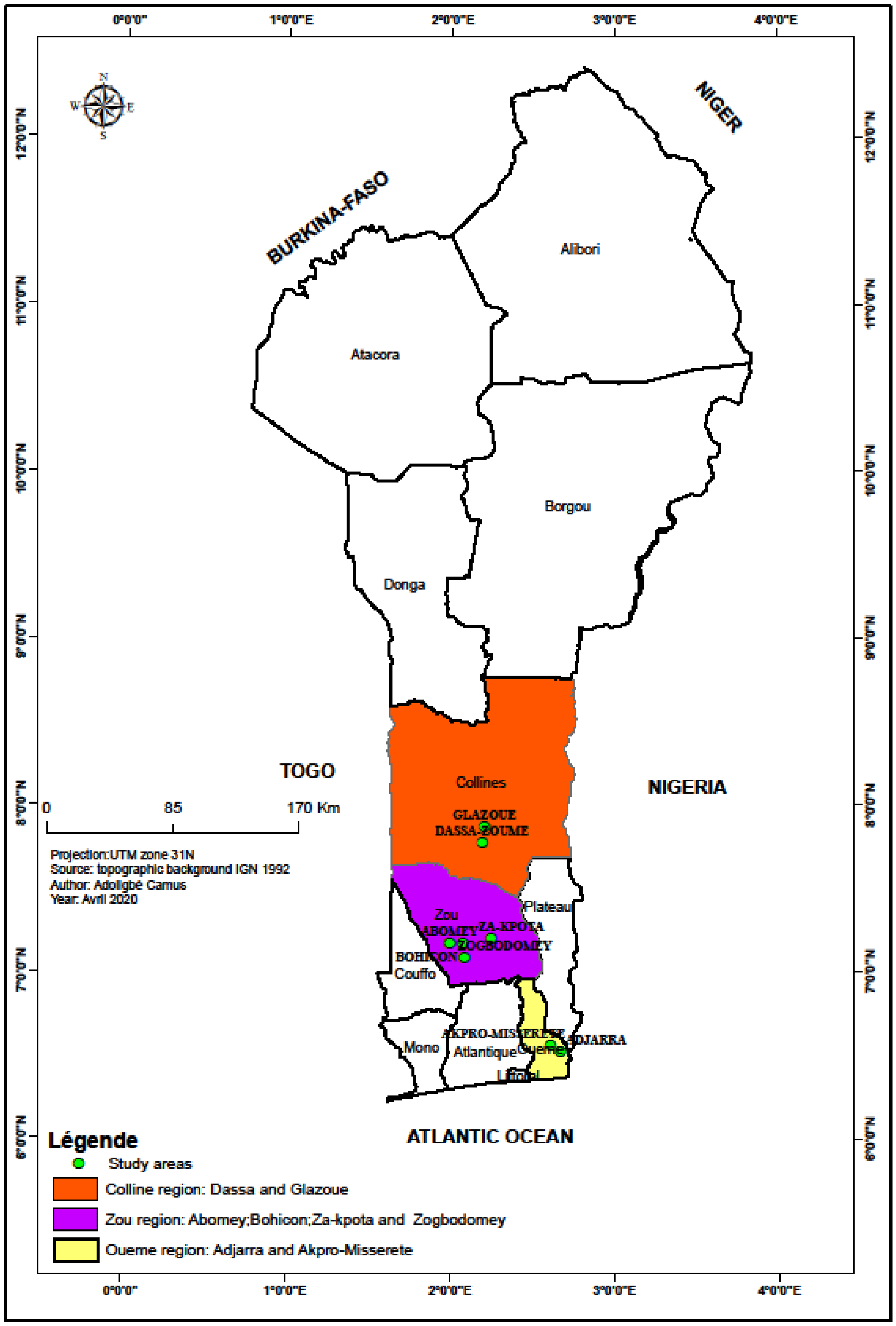


Figure 2. Map of Benin showing the study areas.

homestead of the respondents, ownership of chickens in the household, flock owner main activity, method of breeding stock acquisition, reason for keeping indigenous chicken and production objectives.

Table 2 shows that in general indigenous chicken was mainly owned by the household head (49 %). Also, majority of the respondents interviewed were crop farmers (26%); however, the respondents' main activity varies from region to region (Table 2). Therefore, majority of the respondents were animal farmers in the region of Zou (50%), crop farmers in the region of Colline (45%) and tradesman in the region of Oueme (33%). As shown in Tables 2 and 3, breeding stocks were mainly purchased (97%) and indigenous chickens were raised mostly to be sold (98%). Production of indigenous chicken was mainly driven by the fact that they are less demanding in terms of inputs (95%). However, their meat quality and the market demand were pointed out by a

Table 1. Demographic distribution of gender and instruction level of the respondents.

Variables	Parameter	Collines (92)			Ouémé (91)			Zou (86)			Total (269)		
		N	%	ME	N	%	ME	N	%	ME	N	%	ME
Gender	Male	63	68.48^{a}	9.49	56	61.54^{a}	10	51	59.30^{a}	10.38	170	63.20^{a}	5.76
	Female	29	31.52^{b}	9.49	35	38.46^{b}	10	35	40.70^{b}	10.38	99	36.80^{b}	5.76
Instruction level	Primary	38	41.30^{a}	10.06	30	32.97^{a}	9.66	55	63.95^{a}	10.15	123	45.72^{a}	5.95
	Illiterate	28	30.43^{ab}	9.4	39	42.86^{a}	10.17	5	5.81^{c}	4.95	72	26.77^{b}	5.29
	Secondary	24	26.09^{b}	8.97	20	21.98^{b}	8.51	25	29.07^{b}	9.6	69	25.65^{b}	5.22
	Tertiary	2	2.17^{c}	2.98	2	2.20^{c}	3.01	1	1.16^{c}	2.27	5	1.86^{c}	1.61

N, observed number of cases for each modality at the different location; % , Relative frequency ; ME, Margin of error . Frequencies within column with different letter are significantly (P<0.05) different according to z-test.

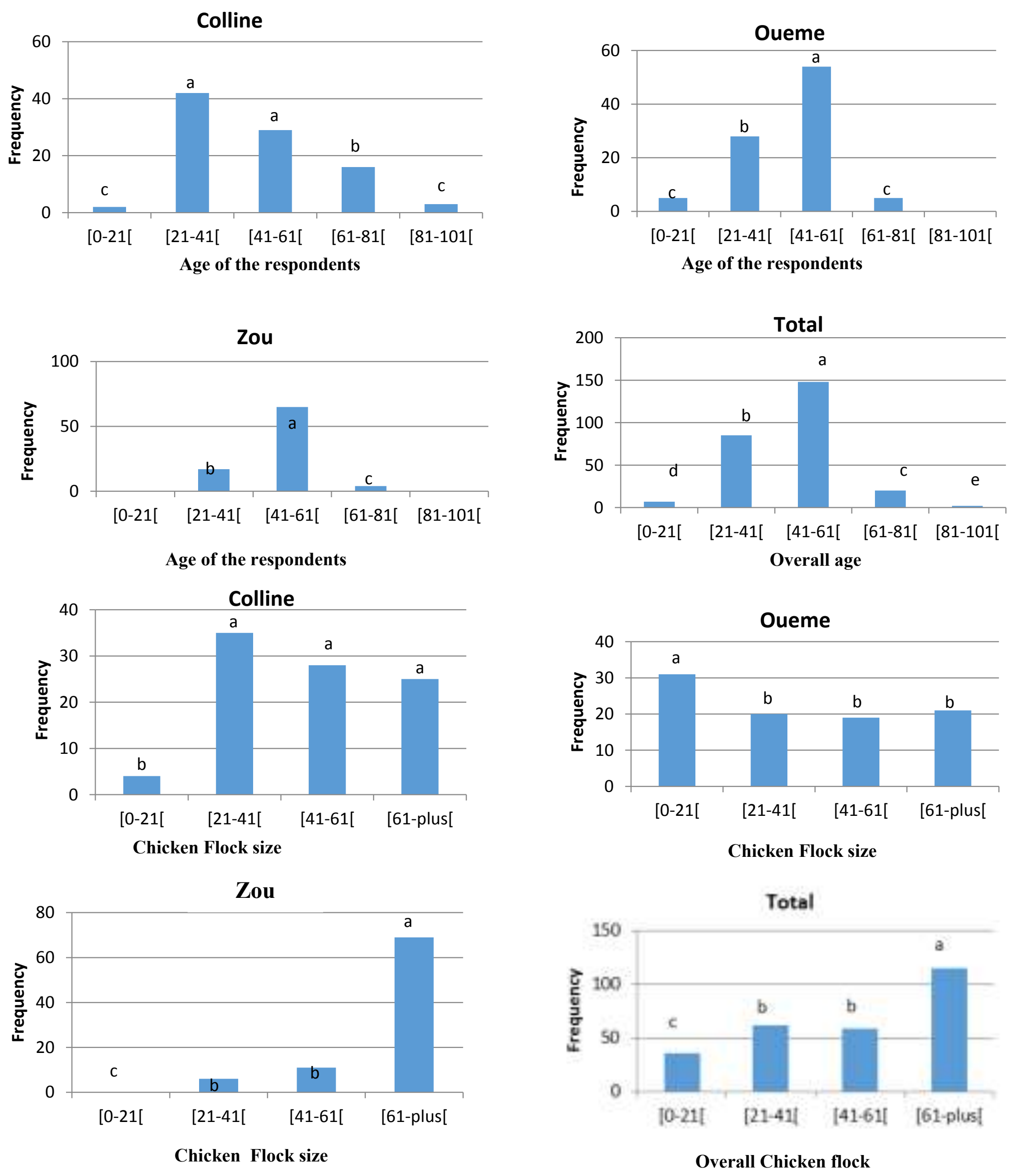


Figure 3. Respondents age structure and chicken flock size (Relative frequency). Rectangle followed by different superscripts show the presence of significant differences (p<0.05)

Table 2. Demographic distribution of ownership, main activity, and method of acquisition.

Variables		Colline (92)			Oueme (91)			Zou (86)			Total (269)		
		N	%	ME	N	%	ME	N	%	ME	N	%	ME
Ownership	Household head	42	45.65^{a}	10.18	49	53.85^{a}	10.24	42	48.84^{a}	10.56	133	49.44^{a}	5.97
	Household head and spouse	10	10.87^{bc}	6.36	0	0.00^{d}	0	10	11.63^{c}	6.78	10	3.72^{cd}	2.26
	Household head, spouse and son	4	4.35^{cd}	4.17	0	0.00^{d}	0	0	0.00^{e}	0	4	1.49^{d}	1.45
	Household head and son	4	4.35^{cd}	4.17	1	1.10^{d}	2.14	0	0.00^{e}	0	15	5.576^{c}	2.74
	In a foster care	7	7.61^{bc}	5.42	0	0.00^{d}	0	0	0.00^{e}	0	7	2.6^{cd}	1.9
	Spouse	16	17.39^{b}	7.75	31	34.07^{b}	9.74	2	2.33^{de}	3.19	49	18.22^{b}	4.61
	Spouse and son	7	7.61^{bc}	5.42	0	0.00^{d}	0	5	5.81^{cd}	4.95	12	4.46^{cd}	2.47
	Son	2	2.17^{d}	2.98	10	10.99^{c}	6.43	27	31.40^{b}	9.81	39	14.49^{b}	4.21
Main activity	Crop farmer	41	44.57^{a}	10.16	18	19.78^{b}	8.18	11	12.79^{c}	7.06	70	26.02^{a}	5.24
	Trader	17	18.48^{bc}	7.93	23	25.27^{ab}	8.93	25	29.07^{b}	9.6	65	24.16^{a}	5.12
	Animal farmer	9	9.78^{c}	6.07	2	2.20^{c}	3.01	43	50.00^{a}	10.57	54	20.07^{a}	4.79
	Craftman	21	22.83^{b}	8.58	30	32.97^{a}	9.66	0	0.00^{d}	0	51	18.96^{a}	4.68
	House keeper	1	1.09^{d}	2.12	13	14.29b	7.19	2	2.33d	3.19	16	5.95b	2.83
	Civil servant	2	2.17^{d}	2.98	4	4.40^{c}	4.21	3	3.49^{d}	3.88	9	3.35^{bc}	2.15
	Mixed crop-animal farmer	1	1.09^{d}	2.12	1	1.10^{c}	2.14	2	2.33^{d}	3.19	4	1.49^{c}	1.45
Method of acquisition	Gifting	4	4.35^{b}	4.17	5	5.49^{b}	4.68	0	0.00^{b}	0	9	3.35^{b}	2.15
	Purchasing	86	93.48^{a}	5.05	89	97.8^{a}	3.01	86	100.00^{a}	0	261	97.03^{a}	2.03
	Inheritance	4	4.35^{b}	4.17	5	5.49^{b}	4.68	0	0.00^{b}	0	9	3.35^{b}	2.15
	Fostering	2	2.17^{b}	2.98	1	1.09^{b}	2.14	0	0.00^{b}	0	3	1.12^{b}	1.25

N, observed number of cases for each modality at the different location; % , Relative frequency ; ME, Margin of error . Frequencies within column with different letter are significantly ($P<0.05$) different according to z-test

significant number of respondents from Oueme (99 and 98%, respectively) and Zou region (100 and 95%, respectively) (Table 3).

Technological factors that influence indigenous chicken production

These include animal excreta management, weaning practice, main rearing system, flock size, average number of eggs per clutch, and major challenges faced by indigenous chicken farmers. The study showed that there were three major production systems, namely free-range extensive system, backyard extensive or semi-intensive system (Table 4). The common production system used in general was mostly the free-range extensive system (70%). However, in the region of Zou, backyard extensive system was predominant (67%). Generally, most chicken farmers used the animal excreta as a fertilizer (55%), but in the region of Colline there was no management of the excreta in the majority of cases (79%). Chicks weaning practice was not common among the farmers except in Zou where majority of the respondents (67%) practice chicks weaning. Average number of eggs per clutch was 11 (Table 5) with a slightly higher number at Colline (12) and a lower number at Zou (10). The chicken flock size was higher than 60, for the majority of respondents. Similar trend was observed in the region of Zou while majority of the farmers at Colline owned in between 21 and 40 chickens and the majority of farmers in the region of Oueme less than 21 (Figure 3). With regards to the major challenges cited by indigenous chicken farmers,

Table 3. Demographic distribution of reasons and objectives of production given by the respondents.

Variables		Collines (92)			Oueme (91)			Zou (86)			Total (269)		
		N	%	ME	N	%	ME	N	%	ME	N	%	ME
Reasons of production	Meat quality	22	23.91[b]	8.72	90	98.90[a]	2.14	86	100.00[a]	0	198	73.61[b]	5.27
	Market demand	12	13.04[bc]	6.88	89	97.80[a]	3.01	82	95.35[b]	4.45	183	68.03[b]	5.57
	Resistance to disease	2	2.17[d]	2.98	58	63.74[b]	9.88	2	2.33[c]	3.19	62	23.05[d]	5.03
	Tradition	7	7.61[cd]	5.42	4	4.40[c]	4.21	78	90.70[b]	6.14	89	33.09[c]	5.62
	Less demanding	85	92.39[a]	5.42	89	97.80[a]	3.01	81	94.19[b]	4.95	255	94.80[a]	2.65
Objectives of production	Self-consumption	84	91.3[ab]	5.76	25	27.47[b]	9.17	66	76.74[b]	8.93	175	65.06[b]	5.7
	Selling	91	98.91[a]	2.12	88	96.7[a]	3.67	84	97.67[a]	3.19	263	97.77[a]	1.76
	saving	83	90.22[b]	6.07	84	92.31[a]	5.47	1	1.16[c]	2.27	168	62.45[b]	5.79

N, observed number of cases for each modality at the different location; % , Relative frequency ; ME, Margin of error . Frequencies within column with different letter are significantly (P<0.05) different according to z-test.

Table 4. Demographic distribution of excreta management, weaning practice and production system.

Variables		Collines (92)			Oueme (91)			Zou (86)			Total (269)		
		N	%	ME	N	%	ME	N	%	ME	N	%	ME
Excreta management	Sale	0	0[c]	0	2	2.197[c]	3.01	0	0.00[b]	0	2	0.74[d]	1.03
	Fertiliser	12	13.04[b]	6.88	51	56.04[a]	10.2	86	100.00[a]	0	149	55.39[a]	5.94
	Gifting	6	6.52[bc]	5.05	5	5.49[c]	4.68	0	0.00[b]	0	11	4.09[c]	2.37
	No management	73	79.35[a]	8.27	37	40.66[b]	10.09	0	0.00[b]	0	110	40.89[b]	5.88
Weaning practice	Yes	5	5.43[b]	4.63	15	16.48[b]	7.62	58	67.44[a]	9.9	78	29.00[b]	5.42
	No	87	94.57[a]	4.63	76	83.52[a]	7.62	28	32.56[b]	9.9	191	71.00[a]	5.42
Production system	Free-range extensive	78	84.78[a]	7.34	84	92.31[a]	5.47	27	31.40[b]	9.81	189	70.26[a]	5.46
	Backyard extensive	12	13.05[b]	6.88	2	2.2[bc]	3.01	58	67..44[a]	9.9	72	26.77[b]	6.94
	Small scale intensive	2	2.17[c]	2.98	5	5.49[b]	4.68	1	1.16[c]	2.27	8	2.97[c]	2.03

N, observed number of cases for each modality at the different location; %, Relative frequenc ; ME, Margin of error. Frequencies within column with different letter are significantly (P<0.05) different according to z-test.

Table 5. Descriptive analysis of average number of eggs per clutch.

Variables	Collines		Ouémé		Zou		Total		ANOVA
	Moy	ES	Moy	ES	Moy	ES	Moy	ES	
Mean	12.47[a]	0.37	11.39[b]	0.3	9.98[c]	0.1	10.97[b]	0.18	***

Means with different letter are significantly (P<0.001) different according to anova.

theft rank first, followed by mortality, disease,access to bank loan, investment, and lack of training (Figures 4, 5, 6 and Table 6).

Relationship between variables

Chicken flock size variation has been used as an indicator to assess how different factors affect the performance of the indigenous chicken enterprises in the study areas. From our results (Table 7), gender and the main activity of the chicken farmer significantly influence the flock size. Therefore, male chicken farmers have larger flocks than female chicken farmers. On the other hand, animal farmers own more chickens than crop farmers, tradesmen, craftsmen, and public servants. Likewise, flock size is larger when weaning is practiced, and excreta is used as fertilizer. There is also a variation in flock size between municipalities with the municipalities of Zogbodomey, Abomey, and Za-kpota having the largest flock sizes. It is worth noting that chicken farmers who mentioned access to bank loan to be their biggest constraint and those who have chosen to rear the indigenous chicken mainly because they can survive in

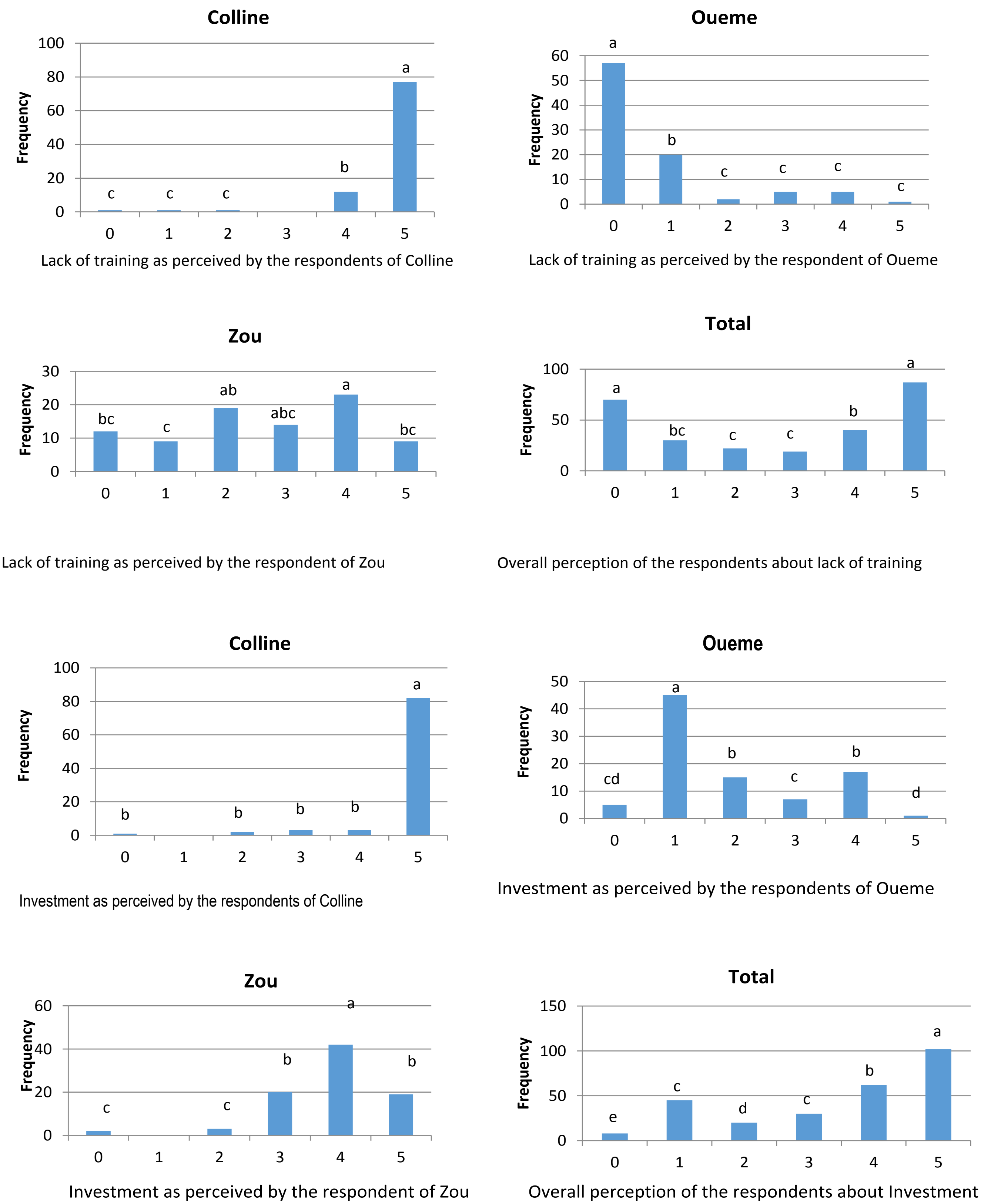


Figure 4. Perception of the respondents about Theft and Access to bank loan as a constraint (Relative frequency. Rectangle followed by different superscripts show the presence of significant differences(p<0.05); 0, 1, 2, 3, 4,5 represent the extent of the constraint as perceive by the respondent: 0=not a constraint, 1=yes at a lower extent, 2= yes at a medium extent, 3= major constraint, 4=one of the biggest constraint, 5=the biggest constraint.

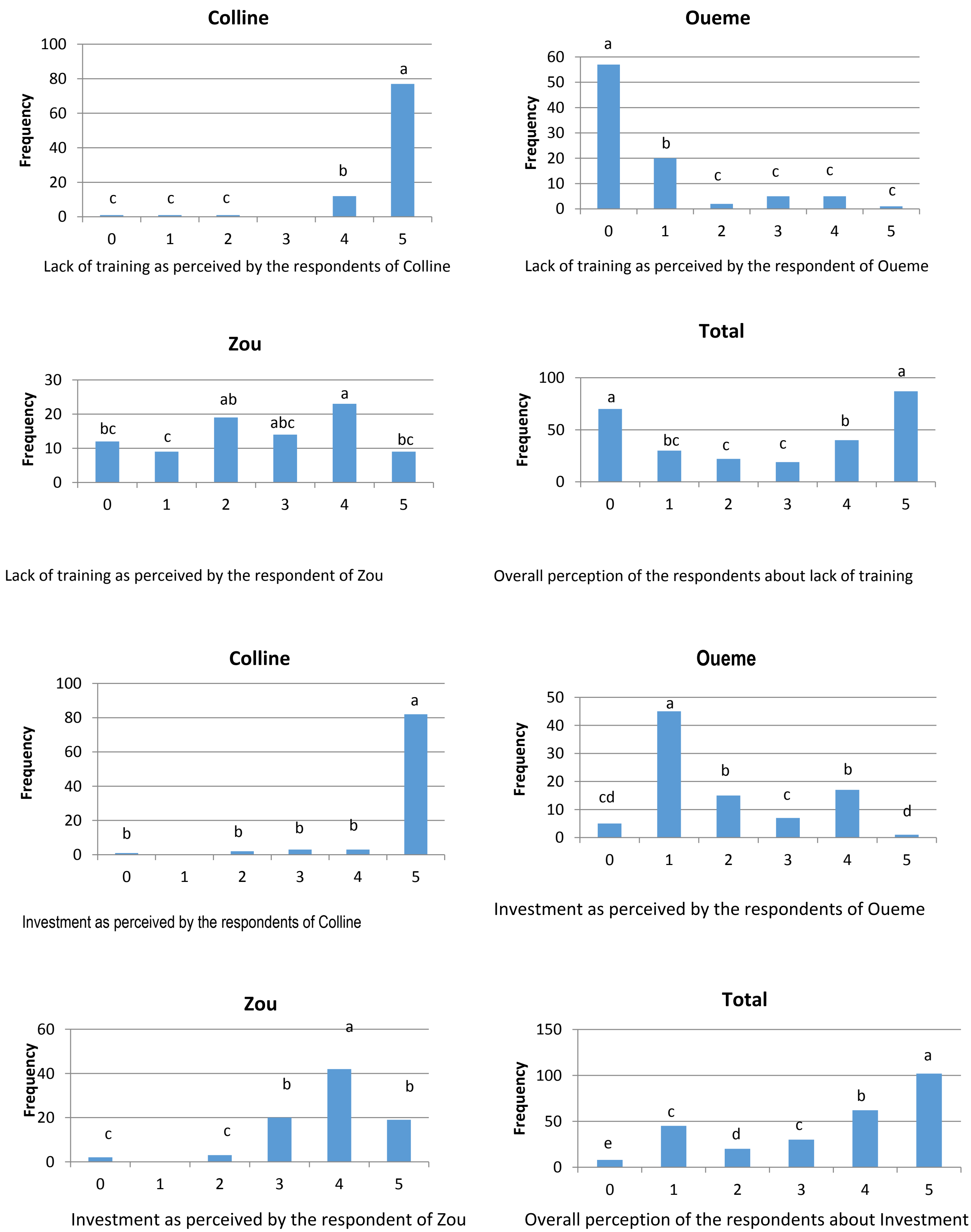


Figure 5. Perception of the respondents about lack of training and Investment as a constraint (Relative frequency). Rectangle followed by different superscripts show the presence of significant differences ($p<0.05$); 0, 1, 2, 3, 4,5 represent the extent of the constraint as perceive by the respondent: 0=not a constraint, 1=yes at a lower extent, 2= yes at a medium extent, 3= major constraint, 4=one of the biggest constraint, 5=the biggest constraint.

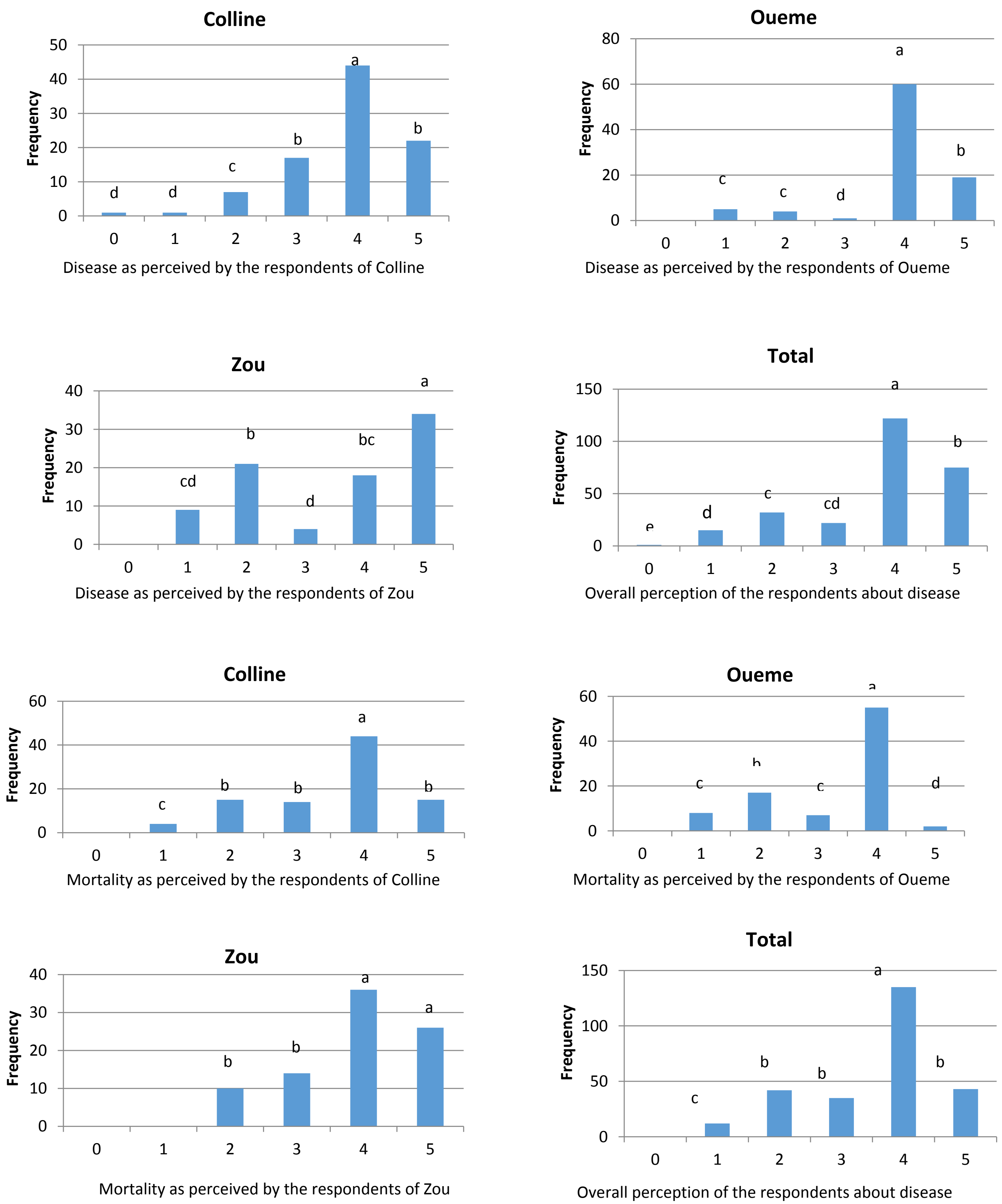


Figure 6. Perception of the respondents about disease and mortality as a constraint (Relative frequency). Rectangle followed by different superscripts show the presence of significant differences ($p<0.05$); 0, 1, 2, 3, 4,5 represent the extent of the constraint as perceive by the respondent: 0=not a constraint, 1=yes at a lower extent, 2= yes at a medium extent, 3= major constraint, 4=one of the biggest constraint, 5=the biggest constraint.

Table 6. Constraints as rated by respondents.

Variables	Theft		Mortality		Investment		Lack of training		Accces to bank loan		disease		p-value
	%	ME	%	ME	%	ME	%	ME	%	ME	%	ME	
0	1.12[bc]	1.26	0.00[c]	0.00	3.00[b]	2.04	26.22[a]	5.28	3.37[b]	2.16	0.37[c]	0.73	***
1	6.37[c]	2.93	4.49[c]	2.49	16.85[ab]	4.49	11.24[b]	3.79	20.22[a]	4.82	5.62[c]	2.76	***
2	11.61[a]	3.84	15.73[a]	4.37	7.49[b]	3.16	8.24[b]	3.30	13.86 [a]	4.14	11.99[a]	3.90	*
3	15.36[a]	4.32	13.11[a]	4.05	11.24[a]	3.79	7.12[b]	3.08	10.86[a]	3.73	8.24[b]	3.30	*
4	24.72[b]	5.17	50.56[a]	6.00	23.22[b]	5.06	14.98[c]	4.28	19.85[bc]	4.78	45.69[a]	5.98	***
5	40.82[a]	5.90	16.10[d]	4.41	38.20[ab]	5.83	32.58[bc]	5.62	32.21[bc]	5.61	28.09[c]	5.39	***

ME, Margin of error; %, Relative Frequency; Frequencies within line with different letter are significantly (*P<0.05 or *** P<0.001) different according to a Chi-squared test, 0, 1, 2, 3, 4,5 represent the extent of the constraint as perceive by the respondent: 0=not a constraint, 1=yes at a lower extent, 2= yes at a medium extent, 3= major constraint, 4=one of the biggest constraint, 5=the biggest constraint.

Table 7. One-way analysis of variance of municipality, gender main activity, weaning, reason for local chicken rearing, and major constraint as predictor variables for flock size.

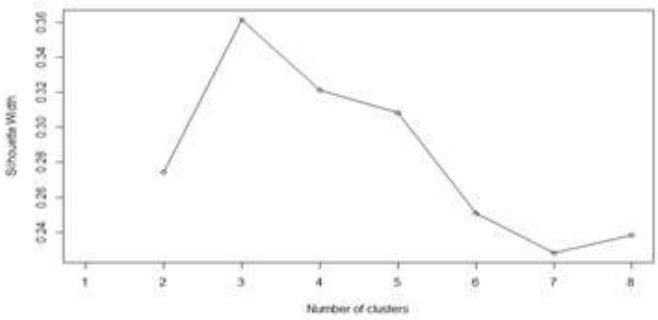
Variable		Mean difference	Significance	95% confidence interval	
				Lower bound	Upper bound
Municipality	Zogbodomey-za-kpota	-129.733	0.000	-212.584	-46.882
	Zogbodomey-abomey	-103.567	0.002	-181.067	-26.067
	Zogbodomey-glazoue	67.176	0.031	3.488	130.864
	Zogbodomey-bohicon	91.850	0.000	45.535	138.165
	Glazoue-adjarra	113.831	0.000	60.399	167.262
	Glazoue-dassa	110.992	0.000	59.437	162.547
	Misserete-glazoue	-105.993	0.000	-160.472	-51.515
	Bohicon-adjarra	89.157	0.000	58.435	119.879
	Dassa-bohicon	-86.318	0.000	-113.646	-58.990
	Misserete-bohicon	-81.319	0.000	-113.828	-48.810
Gender	Male-female	26.284	0.000	13.218	39.350
Main activity	Animal farming-plant farming	34.085	0.010	5.026	63.144
	Animal farming-craftsman	41.154	0.002	9.666	72.642
	Animal farming-tradesman	52.619	0.000	23.077	82.160
	Public servant-Animal farming	-69.589	0.007	-127.354	-11.824
Weaning	Yes-No	29.327	0.000	15.440	43.215
Reason for local chicken rearing: less onerous	Yes-No	-29.581	0.041	-57.950	-1.212
Major constraint: access to bank loan	5-3	-32.782	0.021	-62.417	-3.147

harsh environment with limited food and water resources have fewer animals.

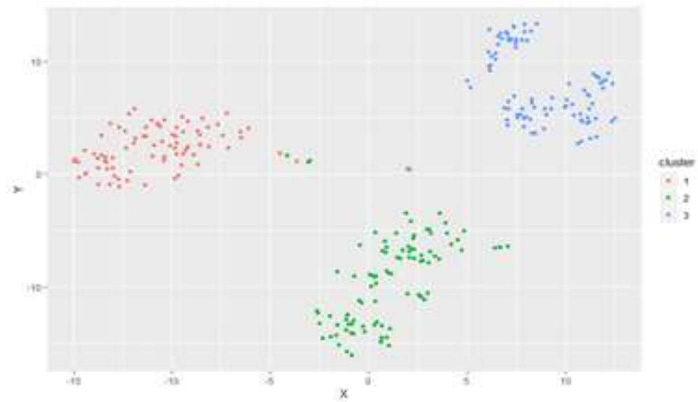
Clustering

A Gower distance analysis was applied to 11 variables to cluster the respondents included in this study in different groups based on their main similarities and differences (Van der Loo, 2017).

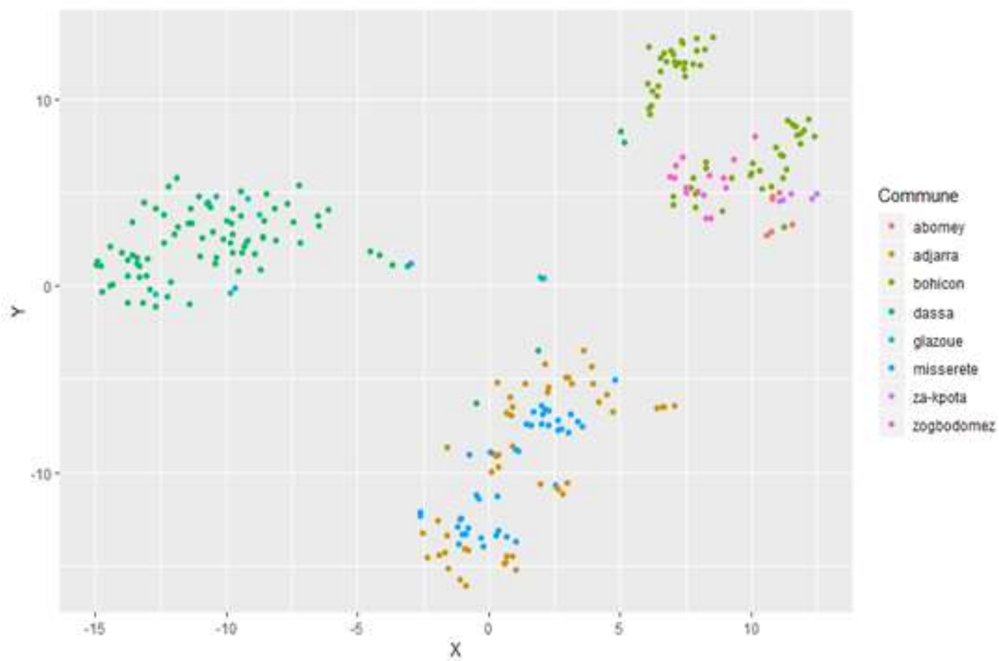
Silhouette analysis showed 3 groups (Figure 7) with 87, 88, and 94 chicken farmers falling into groups 1, 2, and 3, respectively. The majority of the respondents from the municipalities of Dassa and Glazoue of Colline; Bohicon, Zogbodomey, Abomey, and Za-Kpota of Zou; Adjarra and Misserete of Oueme belonged to groups 1, 2 and 3, respectively (Table 8). The majority of the respondents of group 2 were more educated (at least 64% had primary education) and had larger flock sizes (174 on an average) than respondents of group 1(58 on an average)



Silhouette Analysis



Distribution of respondents across clusters (same colour) observed in a lower dimensional space



Distribution of respondents from different municipalities between cluster

Figure 7. Cluster analysis.

and 3(46 on an average). They were mainly animal farmers (50%), adopted backyard extensive system (64%), weaned chicks (59%), and produced organic fertilizer from animal excreta (100%). However, the average number of eggs produced per clutch in this group (10) was lower than what was produced in group 1 (12) and group 3 (11). Although theft was commonly pointed out by respondents from all three groups, the main constraints for chicken production varied from group 1 to group 2. The common reason for raising chickens appears to be its less onerous characteristic. However, meat quality and market demand were also listed by respondents in groups 2 and 3.

DISCUSSION

Our results indicate that local chicken production is widely practiced in the study areas by a diverse group of people, of different gender, age, ethnicity, instruction level and main activity. This can be attributed to many factors including the relatively short reproductive cycle of chickens, the non-existence of cultural and religious ban on chicken meat, the ease to manage as small size and the ability of the indigenous chicken to survive in harsh environment (Ayssiwede et al., 2013; Bessadok et al., 2003). The predominance of male farmers is in agreement with findings by Pafilav (2015) in other parts of Benin.

Table 8. Descriptive analysis of the three different cluster generated via Gower distance.

Variables	Cluster 1		Cluster 2		Cluster 3		Anova/Chi-square
Total number of respondents	87^a		88^a		94^a		NS
Number of respondent from the different municipalities	Dassa	77	Bohicon	60	Adjarra	50	
	Glazoue	10	Zogbodomey	15	Misserete	41	NSTP
			Abomey	5	Dassa	2	
			Za-kpota5	5	Abomey	1	
			Dassa	2			
			Glazoue	1			
Education level of the majority of the respondent (%)	Primary	41	Primary	64	None	41	NSTP
Main activity of the majority of the respondent (%)	Crop farmer	46	Animal farmer	50	Tradesman	33	NSTP
Average number of chicken per cluster	$58.37^b \pm 6.24$		$173.95^a \pm 13.12$		$45.79^b \pm 4.4$		***
Most relevant reason for rearing local poultry breed (descending order)	Less onerous		Meat quality Less onerous Market demand		Meat quality Market demand Less onerous		NSTP
Main Production objectives (descending order)	Selling Saving Self-consumption		Selling Saving Self-consumption		Selling Saving		NSTP
Most relevant breeding system (%)	Free range extensive system	87	Backyard extensive system	64	Free range extensive system	91	NSTP
Poultry manure management (%)	Use as fertilizer	13^c	Use as fertilizer	100^a	Use as fertilizer	56^b	***
Weaning practice (%)	Yes	3^c	Yes	59^a	Yes	16^b	***
Mean of number of egg produce per clutch	$12.48^a \pm 0.37$		$9.98^c \pm 0.1$		$11.38^b \pm 0.3$		***
Major constraints (descending order)	Limited investment Lack of training No access to bank loan Animal theft		Mortality Limited investment Disease Animal theft		Disease Animal theft Mortality		NSTP

Means with different letter are significantly (P<0.001) different according to anova for mean and chi-square for percentage; NS=non-significant; NSTP= no statistical test was performed.

However, reports from other African countries point to the contrary where women are key stakeholders in local chicken farming (Mogesse, 2007; Ahlers et al., 2009; Guèye, 2009; Yusuf et al., 2014; Haoua et al., 2015). Actually, gender-based division of roles and responsibilities in Benin rural society implies that women are often in-charge of the sale of the family enterprise products on the market while men as a household head have the ownership of the enterprise and have full control over production and the resulting profit. This is quite similar to the findings of

Mahoro et al*.* (2017) in Rwanda, who reported that the majority of respondents in their study were males (62%) but women (78%) were highly responsible for local chicken management activities. The proportion of illiterates (27%) in this study is higher than that reported by Murekefu (2013) for the district of Vihiga in Kenya (1%). This implies the unlikelihood for households to positively take up innovative and good poultry management practices and agricultural extension education. The results of the survey conducted by Kawsar et al*.* (2013) in Bangladesh showed that all poultry farm owners were educated, and the sizes of the flock increased in step with level of education. Consistent with this, Chowdhury et al*.* (2009) showed that 52% of the small-scale broiler farmers who reared smaller flocks in their study had only a primary level of education and suggested that small and medium enterprise farmers must have at least Junior School Certificate and they should be trained on poultry science and technology before allowing/registering for commercial poultry farming. Our clusters analysis agrees with these findings. Respondents from cluster 1 and 2 are more educated than respondents from cluster 3 and had larger flock size.

Our study showed that except from Zou where the majority of respondents rear their animals in a semi-confined system, the birds in the other areas of the study were mostly reared on free-range. This is in agreement with previous reports across other African countries (Ndegwa et al*.,* 2014; Haoua et al*.,* 2015; Mahoro et al*.,* 2017; Assefa et al*.,* 2019). The adoption of the free-range rearing system by a significant number of local chicken farmers could be interpreted in different ways. In fact, we found a relationship between the breeding system, reason for rearing the indigenous chicken, production objectives, and the main activity of the rural chicken farmers. Communities with semi-intensive production systems mainly raise local chicken to meet the market demand and for their own consumption. This implies that local chicken raising is their primary income source and explains why they are more eager to apply improved management practices and keep larger flock. However, in the free-range system dominated communities, the indigenous chickens were kept mostly because of their ability to tolerate the harsh environmental condition and poor management practices. This implies that these communities were not really willing to keep breeds that require too much investment and care or they had low level of income to meet their own basic needs, hence, investing in chicken production is not their first priority. The respondents in the free-range system dominated communities did animal farming as a secondary activity and the products were either sold on the local market or used as saving. Hence, time allocation to their main activity could also be one of the reasons that prevent them to intensify the chicken farming activity. Okeno et al*.* (2012) reported that utilization of native chickens in their current genetic merit and production environment is more profitable under free range system and semi-intensive system but not economically viable under intensive system. It was proven that changes in traditional management practices can improve the performance of native chicken and thus contribute to household incomes increase per year (Ondwasy et al*.,* 2006; Sarkar and Golam, 2009). Although these findings are research-based evidence, we believe that intervention to improve production in the traditional system should be community-specific and predetermined. To be sustainable, the perceived needs and interest of the communities must be considered while proposing various recommendations. In our case, we would recommend for farmers of group 1 and group 3 (the free-range dominated groups) to include protein and vitamin supplements from easy growing indigenous plant leaves such as *Moringa oleifera* and *Azolla filiculoides* to balance chicken diets particularly for the young chicks. Assefa et al*.* (2019) have shown that frequent supplementation of baby chicks is important to enhance their growth until they reach the age of full scavenging potential. Farmers should be trained and encouraged to provide habitat for their animal using local materials. Endemic disease control must be strengthened with the free supply of vaccines. To this regard, recent effort of the Government of Benin to provide free immunization against new castle disease in the rural communities must be encouraged (Procad, 2019). There is a need to organize farmers in groups where they can mobilize their limited resources to carry out activities like group-based vaccination and purchase of inputs such as drugs to save on cost and ensure proper vaccination timing. Finally, it is necessary to insist on improving education and skills of these farmers, particularly farmers of group 3, for a successful implementation of the improved practices. For the farmers of group 2 (semi-intensive dominated group), much more could be done to improve incomes and productivity. Majority of these respondents are already familiar with chicks weaning. But additionally, they need to learn simultaneous hatching of hens to plan production to meet seasonal demand and increase profits but also to make planning for vaccinations easier. In general, we propose the use of solar powered incubators to enable such farmers to hatch more eggs. Their ability to select best laying hen, broody hen and strong cock to make their breeding stock as well as their ability to build housing with local materials that provide the chicken better living condition and protection against the predators should be upgraded through tailor-made training programmes. Additionally, they should be taught to keep record for a better assessment of their economics on farming.

Our study revealed a significant influence of gender, main activity, weaning practice, reason for breeding the local chicken, and major constraint on flock size. We cannot see any reason for female farmers holding much smaller chicken flocks than male farmer except the existence of a gender gap due to social norm. In Benin and Togo, it is common to see men keeping species such as guinea fowl, turkeys, and pigeons. Some farmers that keep these types of poultry and large flock size of birds have certain prestigious value (Thomsen et al., 2005; Kryger et al., 2010). Nelson et al. (2012) reported that inequality in livestock holdings was particularly acute in Bangladesh, Ghana, and Nigeria, where male holdings are more than three times larger than those of female-headed households. Farms run by female-headed households have less labour available for farm work because these households are typically smaller and have fewer working-age adult members and because women have heavy and unpaid household duties that take them away from more productive activities. However, according to Alemayehu et al., (2018), women dominate chicken's ownership in developing countries. This is due to the low investment nature of chicken farming compared to other livestock enterprises. For example, Ahlers et al. (2009) indicated that it is a common practice in sub-Saharan Africa that indigenous chickens are owned and managed by women and children and female-headed households. Whereas men may assist in the construction of housing (night shelters for the animals) and in some localities in bringing birds and eggs to the market, women and children are, as a general rule, the ones who feed and water the birds, clean the housing and apply treatments (Mutombo 2014; Patbandha et al., 2016). Nduthu (2015) mentioned that any development schemes which aim to improve and promote the chicken sectors to be sustainable should not underestimate the contributions and roles of women. Poultry production enterprise is a potential area for women's groups to: harness income, create job opportunities, improve quality of life, investing not only in food diversity, but also in greater access to health, hygiene and access to education for children in rural communities (Nduthu, 2015; Villanueva-Cabezas, 2018).

Therefore, we are tempted to recommend women empowerment as one of the strategies to improve local chicken production in the study areas. However, most of our respondents (men and women) adopted free-range extensive system and seem not to aspire to become full-time chicken farmers. Just like in the case of local chicken farmers in India, they raise chickens as a secondary activity to alleviate food insecurity, as a mean of social engagement in their communities, or to access fast cash when unexpected costs appear (Kumar et al., 2019). Therefore, to have a successful implementation of women empowerment strategies a search for what approach to adopt should be conducted using a reliable and adapted tool such as the Women's Empowerment in Livestock Index (WELI) developed by Galiè et al. (2019).

The practice of chick weaning was associated with larger chicken flocks. Hens that are prevented from brooding their own chicks will start to lay again more quickly after just 21 days, instead of the usual three months (FAO, 2010). Though this method leads to high production of indigenous poultry, it should be taken cautiously as extra measure are needed to boost chick survival rate. These include balanced feeding, sanitary measures and a brooder to ensure weaned chick survival. For the respondent that might not be able to afford it we would recommend a late weaning. They can wean their chicks at around 4 weeks of age when the chicks had acquired immunity against common disease and the ability to cope with harsh conditions. This will allow them to circumvent the requirement for raising day old chicks while reducing the inter-clutch interval. Unsurprisingly, animal farmers held much more chicken than any other respondents since they have to develop their main activity in order to get sufficient income to meet out their needs. Respondents who rear the local chickens because they are less demanding had much small scale chicken flocks than the rest. Consistent with our previous insight, these respondents belong to majority that rear the local chickens in scavenging and are less motivated to intensify their chickens breeding activity. Respondents who perceived access to bank loan as their biggest constraint have fewer chickens. This indicates that facilitating access to credit for the farmers willing to take up such activity would play an important role in boosting indigenous chicken productivity.

The performance of the respondents falling in group 2 stress the need to promote best management practices among the indigenous poultry farmers. However, the average number of eggs per clutch obtained in this group was the lowest. This highlights the limitation of the semi-confined breeding system, particularly for large scale flocks, when dietary requirements are not efficiently met. There is a clear relationship between egg production and nutrient intake. Wanjugu (2013) reported that the size and productivity of the village flock ultimately depend on the human population and its household waste and crop residues, and on the availability of other feed resources obtained from scavenging.

Previous researches have discussed different ways to improve the performance of native chickens. For instance, Abdelqader et al. (2007) reported that there was a significant improvement in hatchability, survivability, flock size, number of clutches, egg weight, and egg mass of native fowl of Jordan with improving the management system alone. Bahmanimehr (2012) claimed that body weight and egg weight improvement of Iranian native chickens can be achieved through selection on the basis that breeding value recorded moderate to high heritability estimates. Effects of crossbreeding of exotic chicken with indigenous chicken were reported in the literature with respect to different traits (Chatterjee et al., 2007; Khan, 2008; Magothe et al., 2012). It was observed that many of the major economic traits improved in the crossbreds compared to native chickens indicating that this is one of the tools to improve the performance of indigenous chickens.

However, from our study, the genetic potential of the indigenous chickens was not pointed as a major constraint. Moreover, the quality of the rural chicken meat and the market demand for the product together with their adaptive traits and ease of keeping justifies the sustainable use and conservation of local chicken ecotypes. Hence, crossbreeding may not be the best option to achieve local chicken performance improvement in the targeted areas. But, changes in traditional management practices combined with the development of breeding programs focusing on within breed selection and a better organization of the local chicken market as suggested by Ndirangu et al. (2015) will certainly yield better results. This would help to maintain the indigenous chicken unique attributes which are appreciated by producers and consumers and avoid genetic dilution and contribute to their conservation.

Conclusion

This study provides a basis for the improvement, sustainable use, and conservation of local chicken production in southern and central Benin. Our results showed that indigenous poultry farmers can be divided into three main clusters whose management practices, production objectives and reason for keeping the indigenous chicken breed are well determined and interrelated. To be efficient, any intervention to improve production should respect this interrelationship to avoid breaking the delicate balance form by these communities, their chicken, and their environment. A combination of improved management practices with suitable selection program of superior breeding stock is recommended to ensure a more productive and sustainable local chicken industry. But there is a need to adapt the management interventions to appropriate to the socio-economic reality of each community. Like in the case of egg producers from hybrid breed in Benin, there is a need to develop countrywide an indigenous chicken value chain to allow farmers to be able to produce and sell chicken in an environment they fetch higher profit. Local authorities should offer incentives and bring local chicken farmers together as a group and provide infrastructure to support poultry processing marketing (slaughterhouses, processing, and cold storage). Rural women empowerment might be a useful mean to boost the local chicken industry. But proper study is needed to look for the best approach to this end. Although expensive, the creation of a breeding index based on traits of interest would also be an interesting approach.

Innovations like the development of a hatchery for day-old chicks of local chicken could emerge as the indigenous poultry value chain develops further. Service providers will benefit by offering a wider range of services including poultry slaughter facilities. The quality and range of poultry products will increase through value addition. Indeed, the export of local chicken meat to the Nigerian market, for instance, will boost the industry with employment opportunities emerging as the local chicken value chain becomes fully operational.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Metagenomic assessment of the rumen resistome, mobilome and stress response genes in smallholder dairy cattle in Kenya

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Smallholder dairy cattle rumen microbiotas are subjected to a wide range of antimicrobials as well as sudden fluctuations in diets. As such, they develop an enormous reservoir of resistant genes, mobilome and stress response genes. However, information on metagenomic reactions to such dietary variations, especially for cattle reared in the tropics, remains largely unexplored. This meta-analysis was conducted to assess if antibiotic and toxic compound resistance genes (ARGs), stress response genes and bacterial phages, prophages and transposable element genes were present, and to what extent, in three dairy cattle genotypes (Friesian, FriesianXJersey crossbreed, Jersey) reared in a farm that practiced judicious use of antimicrobials. Potential bacterial hosts to these genes were also explored. The rumen metagenomes generated from Next Generation Sequencing (NGS) technology were analyzed using MG-RAST. According to the results stress reaction, resistance and mobilome genes were present in similar amounts in all the three genotypes. Cobalt-zinc-cadmium resistance, fluoroquinolone resistance, methicillin resistance in Staphylococci and multidrug resistance efflux pumps were the most abundant resistant genes and were spread across 20, 24, 16 and 21 bacterial classes, respectively. Bacteria in charge of phage integration and excision, phages replication and phage packaging were mostly allocated to the phyla Firmicutes, Bacteroides and Proteobacteria. Within the stress response genes, metagenomic assembly-based host-tracking analysis identified the extended heat shock dnaK gene cluster as the most abundant genes, while Bacteroides and Clostridium were the principal bacterial hosts. The results show that even with proper use of antimicrobials, the cattle rumen contained an immense distribution of responses to stress, ARGs and mobilome genes distributed in a vast assemblage of hosts. There is also a high correlation between these three functional groups.

Key words: Resistome, metagenome, MG-RAST, Stress genes, mobilome

INTRODUCTION

Ruminants are a mammalian group that includes domestic cattle, sheep, and goats. The importance of domesticated

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ruminants is derived from their capability to change forages into high-quality, high-protein products for human consumption, through rumen fermentation (Ross et al., 2012). The rumen is the ruminant stomach's first chamber, and it has been bestowed upon by nature to harbor a large amount of symbiotic microflora and fauna (Reddy et al., 2014). Smallholder dairy cattle are subjected to a range of diets which mainly consist of lignocellulosic by-products such as cereal straws. These plant products are digested by cattle through microbial processes in the rumen (Kong et al., 2010; Morgavi et al., 2013; Patel et al., 2014). The most important microbes in the rumen are bacteria; this is because they are the predominant group. Additionally, the rumen contains an assortment of archaea, fungi and protozoa (Hespell et al., 1997).

Colonization of the rumen by microbial communities begins within the first 24 h of birth (Jami et al*.,* 2013; Li et al*.,* 2012) and continues to increase in mass and density as the cow is exposed to different diets. However, as the rumen microbes increase, there is also a corresponding increase of the resistance, mobile and stress response genes in the cattle digestive tract. Of more importance are the antimicrobial-resistant (AMR) genes and bacteria that the cattle harbor and that have the potential to spread to humans who consume products from these animals. AMR genes and bacteria are mainly acquired from phylogenetically distant microbiomes in the animals environment (soil, food, water, other animals and or humans) (Westphal-Settele et al., 2018). This genetic transfer is facilitated through horizontal gene transfer of the mobilomes (mobile genetic elements) (Forsberg et al., 2012; Wallau et al., 2018). Besides AMR acquisition, AMR genes have been shown to develop in animals. This has been mainly linked to the antimicrobial usage in the production cycle (Cameron and McAllister, 2016; Penders et al., 2013). Nevertheless, AMR has been reported in non-medicated animals (Chambers et al., 2015; Poole, 2012). This appearance of AMR genes has largely been associated with microbial responses to stresses in dietary changes (Auffret et al., 2017). Nutritional modifications have been shown to trigger a "bloom" of particular microbial communities or increase the abundance of other stress-response genes in the gut microbial population (Keto-Timonen et al., 2016; Shin et al., 2015). It is therefore important to understand the diversity of these resistances and stress response genes in smallholder dairy cattle which are subjected to constant variations in diets. Additionally, since the gastrointestinal tract is an open system which comes across numerous bacteria every day, (Baquero, 2012), an understanding of the mobile genes in this system becomes imperative.

For many years, conventional culture-dependent methods have been used to assess rumen microbial population structure. However, these techniques provide a restricted and biased image of the existing rumen microbial community (de Menezes et al., 2011; Hess et al., 2011; Jami and Mizrahi, 2012). These challenges have been overcome with the advent of metagenomics, a next-generation sequencing (NGS) technique that isolates DNA from the whole community irrespective of individual microbial culturing conditions. Using metagenomics, previous studies have assessed functional selections of all genes that confer resistance, including putative or precursor resistance genes (resistome) (Berendonk et al., 2015; Wright, 2007). The resistome has been postulated to be the cause recognized techniques of resistance, such as modifying an antibiotic target, enabling the cell to efflux antibiotic compound or producing an enzyme capable of disabling active compounds (Gomez-Alvarez et al., 2012). Additionally, applications of metagenomics has revealed a complex network of genetic exchange between bacterial pathogens and environmental reservoirs in antibiotic resistance and stress genes studies (Reddy et al., 2014).

It is therefore imperative that the relationship between the antimicrobial resistance genes, mobile genes and stress response genes be properly investigated especially for animals where proper antimicrobial use is practiced. In addition, breed differences in these genes should be assessed especially at the smallholder farm level. Given the foregoing, the aim of this study was to provide a description of the phylogenetic and functional potential of rumen resistome, mobilome, and stress genes in smallholder dairy cattle reared in the tropics as well as the host bacteria associated with these genes.

MATERIALS AND METHODS

Experimental design and rumen sampling

The experimental animals were maintained at the University of Nairobi's Faculty of Veterinary Medicine farm. Six 3–4 years old healthy dairy cattle were used in this study. The animals were from three genotypes; Friesian (Fri), Jersey (Jer) and Friesian X Jersey cross (FriXJer), with two animals per genotype. Animals were purposefully selected based on their breed, body condition, medical history, and stage of lactation. Prior to the study, all animals were maintained on pasture, which was predominantly kikuyu grass (*Pennisetum clandestinum*) with daily supplementation with dairy meal (a formulated concentrated diet during milking). During the dry seasons, the animals were also offered rhodes grass (*Chloris gayana*) hay, Napier grass (*Pennisetum purpureum*), and maize (*Zea mays*) stover or silage. Additionally, mineral supplement and fresh clean water were given to the cows ad libitum. On the sampling day, individual rumen liquor samples were collected at approximately 0900 h using a flexible stomach tube as previously suggested by Lodge-Ivey et al. (2009). Samples were promptly put on ice, transported to the laboratory and stored at -20°C before metagenome analysis.

DNA extraction, library preparation and Illumina Miseq Sequencing

A homogenized subsample of the rumen liquor samples was

Table 1. Phylogenetic classification at domain level.

Domain[1]	Fri (%)	Fri×Jer (%)	Jer (%)
Bacteria	97.662	98.074	97.846
Eukaryota	1.701	1.349	1.536
Archaea	0.561	0.492	0.554
Viruses	0.077	0.085	0.064
Unclassified	0	0.001	0

[1]Means are based on 2 cows. Fri = Friesian, Jer = Jersey and Fri×Jer = Friesian × Jersey cross.

subjected to DNA isolation using the QIAmp DNA stool mini kit (Qiagen, Valencia, CA). DNA samples were measured on a NanoDrop™ 2000 spectrophotometer (Thermoscientific, USA) and Qubit® 2.0 Fluorometer (Life Technologies Corporation, Grand Island, NY, USA), to assess DNA quantity as previously described by Habimana et al. (2018). Thereafter, libraries were generated using the Nextera XT DNA Library and index kit. The quality and amount of libraries produced were evaluated using the Agilent Bioanalyzer TapeStation 2200 (Agilent Technologies, Santa Clara, USA) with Agilent High Sensitivity DNA Kit (Agilent Technologies), while the quantification was achieved using Qubit® fluorometer (Life Technologies). Quality filtered libraries were sequenced on the Illumina Miseq platform at the Biosciences eastern and central Africa - International Livestock Research Institute, (BecA ILRI) Hub laboratory in Nairobi.

Bioinformatics analysis

Data analyses were performed using the publicly available Metagenome Rapid Annotation Subsystem Technology (MG-RAST) pipeline. The raw sequence information, together with their related quality results (FASTQ format), were used for the optional first quality control (QC) filter to remove duplicate and poor-quality reads. For functional and diversity assessment, the reads that passed the quality filters were subjected to the M5NR database (M5 non-redundant protein database, http://tools.metagenomics.anl.gov/m5nr/) applying an e-value threshold 1.0e-05. The M5NR is a single, searchable, non-redundant database that contains protein sequences and annotations from various sources and their associated tools. In addition, SEED subsystems (Overbeek et al., 2005) (http://www.theseed.org/wiki/Home_of_the_SEED), was used for the functional hierarchical classification applying an e-value threshold of 1.0e-05 (Edwards et al., 2006). The difference in the gene content of resistance to antibiotics and toxic compound, phages and prophages and stress response between different cattle genotypes was quantified using one-way analysis of variance (ANOVA) with Bonferroni adjustment using Genstat version 14 software (Payne, 2011). Significance was established at $P \leq 0.05$. To identify which organisms were associated with the genes allocated to each subcategory, sequence alignment using BLAT incorporated in the MG-RAST database was undertaken.

Ethics statement

This study was approved and performed following the University of Nairobi's Faculty of Veterinary Medicine Animal Care and Use Committee (ACUC) guidelines. Animals were handled by experienced animal health professionals to minimize discomfort and injury.

RESULTS

Taxonomic characterization of the sequencing samples

In this study, genetic diversity and functional capacity of the small holder cattle rumen microbiota were characterized through metagenomic sequences. Supplementary Table S1 summarizes the metagenome information. Illumina Miseq sequencing resulted in a total of 4.85 million reads from all the samples, with different reads length (90-94 bp). At the domain level, data was dominated by bacteria followed by eukaryotes, archaea and viruses (Table 1). Within the eukaryotic sequences, Fungi, Metazoa and Viridiplantae were the most abundant. We hypothesize that this could have been as a result of plant DNA contamination. The phylum level breakdown in our data showed that Bacteroidetes, Firmicutes and Proteobacteria predominated in all the genotypes (Figure 1).

Predicted gene functions of the rumen metagenomes

Sequences annotations using the SEED interface demonstrated the existence of functional protein encoding genes (PEGs). All the metagenomes showed that PEGs belonging to four subsystems namely, carbohydrates (14.47 to 14.87%), protein metabolism (13.7 to 14.38%), clustering-based subsystems (12.68 to 12.81%) and amino acids derivatives (10.17 to 10.57%), were most abundant. The functional classification by the subsystem database also indicated the presence of mobilome, resistome and stress genes as shown in Figure 2. Focusing at specific metabolic pathways, reads assignment to virulence, disease and defence were 1.86, 1.9 and 1.73% in Friesian, FriesianXJersey cross and Jersey, respectively. Genes that coded for Phages, Prophages, Transposable elements and Plasmids accounted for 1.8% in Friesian, 1.93% in FriesianXJersey cross and 1.63% in Jersey. Stress response genes were 1.81, 1.92, 1.63 respectively in the Friesian, FriesianXJersey cross and Jersey cattle genotypes. One-way ANOVA analysis for the three functional systems, revealed that there were no significant differences within

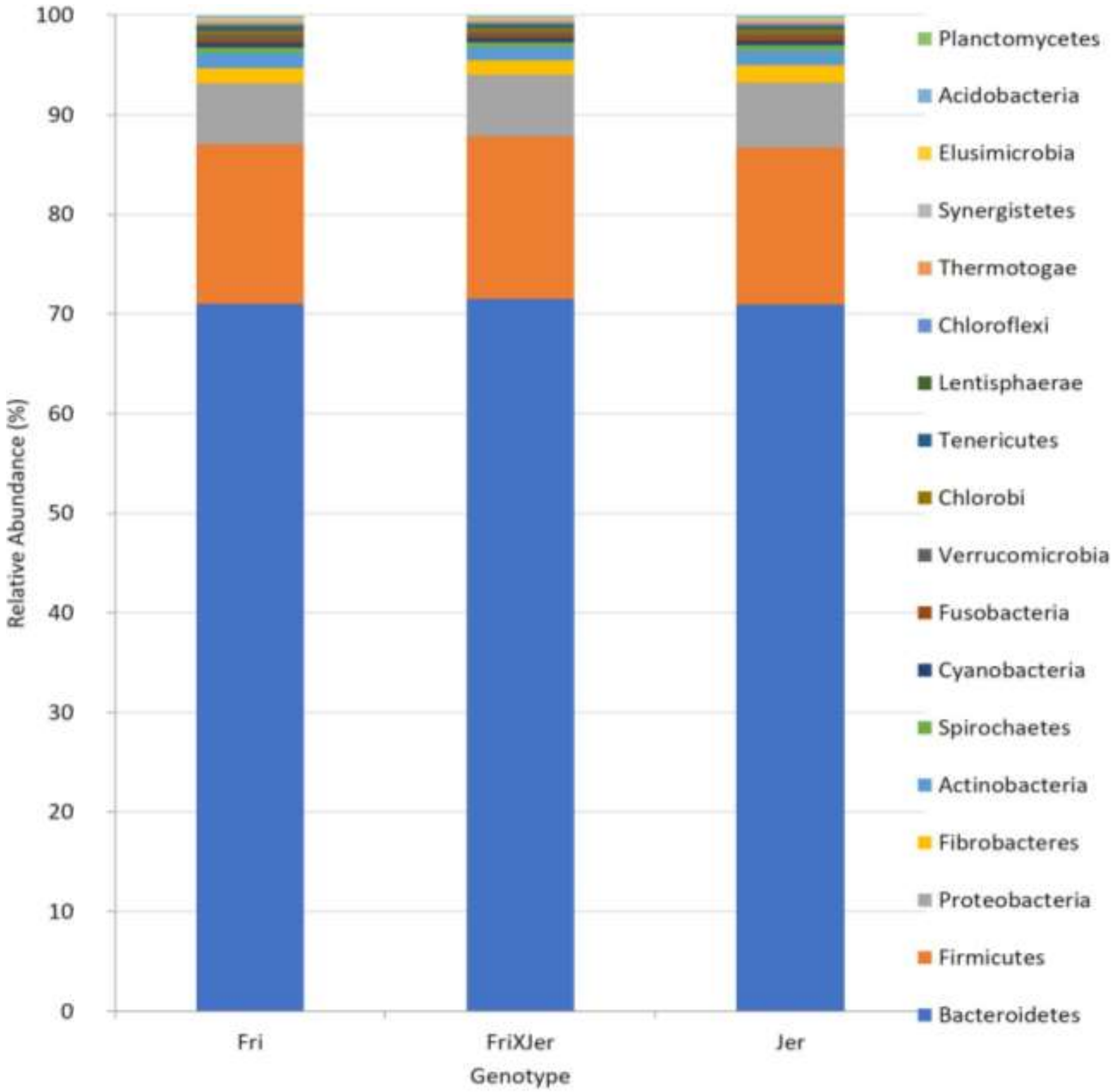

Figure 1. Stacked bar chart of the numerically abundant (relative abundance > 0.1) phyla in the three cattle genotypes. Fri = Friesian, Jer = Jersey and FriXJer = Friesian X Jersey cross.

genotypes; virulence, disease and defence (P = 0.803), Phages, Prophages, Transposable elements (P = 0.283) and Stress response (P = 0.355). Pearson correlation analysis of the resistance genes, mobilome and stress genes indicated a high positive correlation between these functional groups (Table 2).

Resistome analyses

MG-RAST classification of virulence, disease and defence identified seven classes. The most abundant cluster among the seven was resistance to antibiotics and toxic compounds (79.05 to 80.70%). About 12.4 to 14.5% of the genes fell in the category of poorly characterized genes associates with resistance called NULL. Virulence, disease and defence associated proteins involved in adhesion to host cells were in the range of 2.59 to 3.57%. Also, 1.22 to 2.61% of genes were associated with host detection. The other classes included genes responsible for ribosomally synthesized bacteriocins, invasion and intracellular resistance and

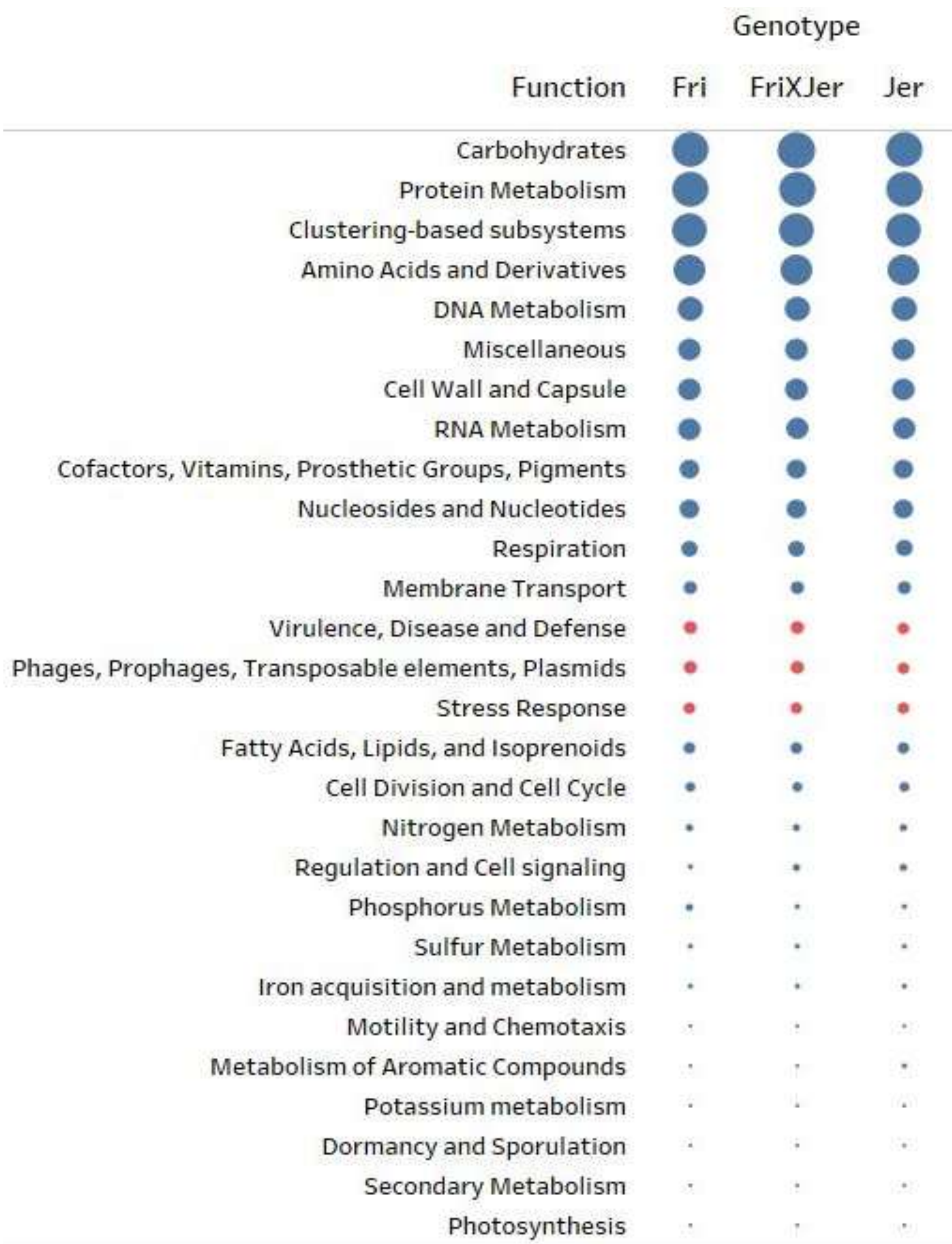

Figure 2. Subsystem level 1 classification of the metagenomes in the three cattle genotypes. Fri = Friesian, Jer = Jersey and FriXJer = Friesian X Jersey cross.

toxins and superantigens (Figure 3). The statistical analysis using One-Way ANOVA showed that only copper homeostasis: copper tolerance was significantly different between all the three genotypes (Table 2). Within the resistance to antibiotics and toxic compounds, the four most abundant subgroups were resistance to fluoroquinolones (28.45 to 29.81%), cobalt-zinc-cadmium resistance (25.75 to 27.12%), multidrug resistance efflux

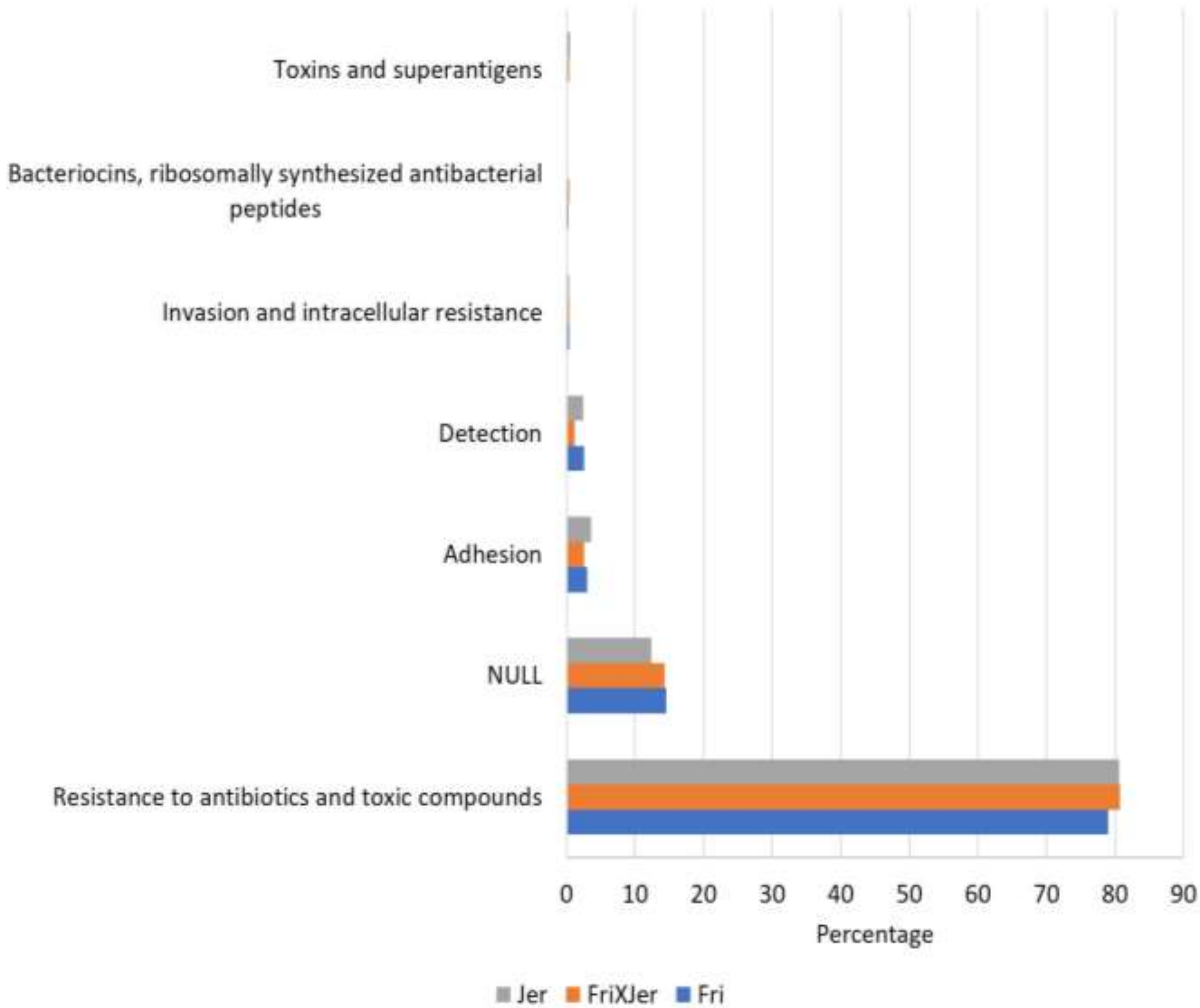

Figure 3. SEED subsystem composition of resistome in the three cattle genotypes. Fri = Friesian, Jer = Jersey and FriXJer = Friesian X Jersey cross.

Table 2. Correlation analysis at level 1 functional classification.

Classification		Resistome	Mobilome	Stress genes
Resistome	Pearson correlation	1	0.999**	0.999**
	Sig. (2-tailed)		0.000	0.000
	N	6	6	6
Mobilome	Pearson correlation	0.999**	1	0.997**
	Sig. (2-tailed)	0.000		0.000
	N	6	6	6
Stress genes	Pearson correlation	0.999**	0.997**	1
	Sig. (2-tailed)	0.000	0.000	
	N	6	6	6

** Correlation is significant at the 0.01 level (2-tailed).

Table 3. Abundance of resistance to antibiotics and toxic compound (RATC) genes from small holder rumen metagenomes (%).

RATC	Fri	FriXJer	Jer	P value
Resistance to fluoroquinolones	**29.812**	**28.903**	**28.453**	0.705
Cobalt-zinc-cadmium resistance	**26.896**	**27.119**	**25.751**	0.907
Multidrug resistance efflux pumps	**14.906**	**16.325**	**15.165**	0.902
Methicillin resistance in Staphylococci	**6.351**	**8.385**	**7.808**	0.162
Arsenic resistance	3.953	4.014	3.679	0.911
BlaR1 family regulatory sensor-transducer disambiguation	3.37	4.55	4.58	0.576
Beta-lactamase	2.592	1.963	2.553	0.273
The mdtABCD multidrug resistance cluster	2.268	2.052	2.252	0.765
Multidrug efflux pump in Campylobacter jejuni (CmeABC operon)	2.009	1.07	0.976	0.556
Cadmium resistance	1.491	1.517	1.276	0.957
Erythromycin resistance	1.426	0.892	1.502	0.647
Copper homeostasis	1.361	1.249	2.027	0.170
MexE-MexF-OprN multidrug efflux system	0.972	0.268	0.676	0.193
Resistance to Vancomycin	0.907	0.892	0.976	0.797
Copper homeostasis: copper tolerance	0.778^{b}	0.178^{a}	0.976^{b}	0.044
Zinc resistance	0.648	0.446	1.051	0.058
Adaptation to d-cysteine	0.13	0.178	0.075	0.825
Bile hydrolysis	0.065	0	0.075	0.465
Mercuric reductase	0.065	0	0	0.465
MexA-MexB-OprM multidrug efflux system	0	0	0.075	0.465
Streptothricin resistance	0	0	0.075	0.465

Bold values indicate the predominance. ^{abc}Indicates significant differences in sample means within breeds.

pumps (14.91 to 16.32%) and methicillin resistance in Staphylococci (6.35 to 8.39%) (Table 3). MG-RAST - BLAT integration revealed that the distribution of specific RATC gene categories is non-random among bacterial taxa. The fluoroquinolone resistance, cobalt-zinc-cadmium resistance, multidrug resistance efflux pumps and Methicillin resistance genes were generally scattered across 24, 20, 21 and 14 bacterial classes, respectively. The predominant phylum in all three genotypes was bacteroidetes followed by firmicutes and proteobacteria (Table 4).

Gene assignments to stress response

In the category of stress response genes, heat shock, oxidative stress and poorly characterized genes associates with stress were predominant in all samples. Conversely, cold shock, detoxification and desiccation stress were the least abundant (Figure 4). Amongst the most principal heat shock system, heat shock dnaK gene cluster extended (33.99 - 37.77%), rubrerythrin (9.26 - 10.51%), regulation of oxidative stress response (8.28 - 9.89%), sigmaB stress response regulation (5.65 - 7.32%) and oxidative stress (6.24 - 6.39%) were predominant in the all treatments samples. No significant differences were observed in all stress response genes between all the three genotypes on One-Way ANOVA (Table 5). Phyla and class-wise affiliation of stress response genes are given in Table 6.

Functional classification of phages, prophages, transposable elements and plasmids

Within the mobilome, phages and prophages, transposable elements and pathogenicity islands had the highest proportions that ranged from; 59.94 to 65.95%, 18.74 to 21.48% and 14.57 to 18.46% respectively. On the other hand, the least amounts were those of gene transfer agent (GTA) (0 to 0.14%), plasmid related functions (0.05 to 0.07%) and poorly characterized functions (0 to 0.16%) (Figure 5). r1t-like streptococcal phages, phage integration and excision, phage replication and phage packaging machinery were the four major subclasses in phages and prophages. The four subclasses accounted for 74-77% in all the metagenomes. Apart from this subclasses, MG-RAST also identified 13 other subclasses (Table 7). On ANOVA, Phage entry and exit (P = 0.023) and Phage nin genes - N-independent survival (P = 0.031) were significantly different in the three genotypes. In both of this function, the FriesianXJersey crossbreed had significantly higher proportions than the other two genotypes. Bacilli were the chief taxa that contributed to the expression of the genes responsible for r1t-like streptococcal phages and phage

Table 4. Bacteria responsible for **A.** fluoroquinolones genes, **B.** Cobolt-zinc-cadmium resistance, **C.** methicillin resistance in Staphylococci and **D**. multidrug resistance efflux pumps genes in the three cattle genotypes.

Phylum	Class	Fri	FriXJer	Jer
A. Resistance to fluoroquinolones				
Actinobacteria	Actinobacteria (class)	1.415	1.684	1.163
Bacteroidetes	Bacteroidia	35.327	37.284	33.747
	Cytophagia	3.291	3.369	2.45
	Flavobacteria	13.406	9.682	7.866
	Sphingobacteria	1.404	2.743	2.342
Chlamydiae	Chlamydiae (class)	1.162	1.049	0.823
Chlorobi	Chlorobia	0.241	0.212	0.465
Deinococcus-Thermus	Deinococci	0	0.212	0
Firmicutes	Bacilli	9.265	9.671	13.687
	Clostridia	12.82	11.153	13.189
	Erysipelotrichi	0	0.212	0.823
	Negativicutes	0.241	0.836	0.947
Fusobacteria	Fusobacteria (class)	0.472	2.097	0.589
Proteobacteria	Alphaproteobacteria	3.981	2.542	1.644
	Betaproteobacteria	1.392	1.059	1.52
	Deltaproteobacteria	3.084	4.417	3.895
	Epsilonproteobacteria	0.461	0	0
	Gammaproteobacteria	3.775	5.879	7.168
Spirochaetes	Spirochaetes (class)	2.335	2.097	3.306
Synergistetes	Synergistia	0	0.212	0
Tenericutes	Mollicutes	4.765	2.955	4.019
Thermotogae	Thermotogae (class)	0.932	0.212	0
Verrucomicrobia	Opitutae	0.23	0.212	0.357
	Verrucomicrobiae	0	0.212	0
	Total (%)	**100**	**100**	**100**
B. Cobalt-zinc-cadmium resistance				
Acidobacteria	Solibacteres	0.245	0.215	0
Actinobacteria	Actinobacteria (class)	4.582	3.017	6.944
Bacteroidetes	Bacteroidia	44.927	44.298	53.247
	Cytophagia	1.935	0.862	1.285
	Flavobacteria	34.299	29.123	18.515
	Sphingobacteria	0.356	0.431	0.99
Chlorobi	Chlorobia	0	1.315	0
Deinococcus-Thermus	Deinococci	0	0	0.296
Elusimicrobia	Elusimicrobia (class)	0	0	0.399
Firmicutes	Bacilli	1.312	0.431	0.296
	Clostridia	3.248	4.139	3.266
	Erysipelotrichi	0.356	0	0
	Negativicutes	1.201	0.215	0.592
Fusobacteria	Fusobacteria (class)	1.667	6.791	2.482
Proteobacteria	Alphaproteobacteria	1.667	0.215	3.857
	Betaproteobacteria	0.735	3.946	3.664
	Deltaproteobacteria	0.845	3.277	0.99
	Gammaproteobacteria	2.379	1.509	3.177
Synergistetes	Synergistia	0	0.215	0

Table 4. Contd.

Verrucomicrobia	Verrucomicrobiae	0.245	0	0
	Total (%)	**100**	**100**	**100**
C. Methicillin resistance in Staphylococci				
Acidobacteria	Solibacteres	1.983	0	0
Actinobacteria	Actinobacteria (class)	0.991	0	0
Bacteroidetes	Bacteroidia	58.693	56.308	57.943
	Cytophagia	15.665	2.759	15.027
	Flavobacteria	7.733	7.789	8.575
	Sphingobacteria	1.124	2.759	4.729
Chlorobi	Chlorobia	0.991	0	0
Firmicutes	Bacilli	4.494	13.225	3.426
	Clostridia	4.363	6.41	3.868
Fusobacteria	Fusobacteria (class)	0	0	1.283
Proteobacteria	Alphaproteobacteria	0.991	0	0.862
	Betaproteobacteria	0.991	3.651	2.564
	Deltaproteobacteria	0	0.69	0.862
	Epsilonproteobacteria	0.991	0.69	0
	Gammaproteobacteria	0.991	5.72	0
Spirochaetes	Spirochaetes (class)	0	0	0.862
	Total (%)	**100**	**100**	**100**
D. Multidrug Resistance Efflux Pumps				
Actinobacteria	Actinobacteria (class)	0.809	0.321	0.524
Bacteroidetes	Bacteroidia	54.382	68.205	59.396
	Cytophagia	0	3.126	0
	Flavobacteria	1.62	1.286	2.084
	Sphingobacteria	0.809	0.321	0.518
Chlorobi	Chlorobia	1.736	3.769	2.607
Chloroflexi	Chloroflexi (class)	0.809	0	0
Cyanobacteria	Gloeobacteria	0.521	0	0
Deferribacteres	Deferribacteres (class)	0	0	0.518
Elusimicrobia	Elusimicrobia (class)	0	0.643	0.518
Firmicutes	Bacilli	0	0	0.518
	Clostridia	7.179	5.378	3.633
	Erysipelotrichi	0.521	0.643	1.041
	Negativicutes	0.405	0.321	0
Proteobacteria	Alphaproteobacteria	3.473	0.964	3.654
	Betaproteobacteria	5.037	5.378	5.208
	Deltaproteobacteria	5.327	0.964	3.12
	Epsilonproteobacteria	2.951	0	1.047
	Gammaproteobacteria	12.973	7.717	14.573
Spirochaetes	Spirochaetes (class)	1.043	0.964	0.518
Synergistetes	Synergistia	0.405	0	0.524
	Total (%)	**100**	**100**	**100**

Fri = Friesian, Jer = Jersey and FriXJer = Friesian X Jersey cross. Percent of genes within each metagenome that are assigned to the listed taxa.

packaging machinery while Bacteroidia was more in phage integration and excision and phage replication.

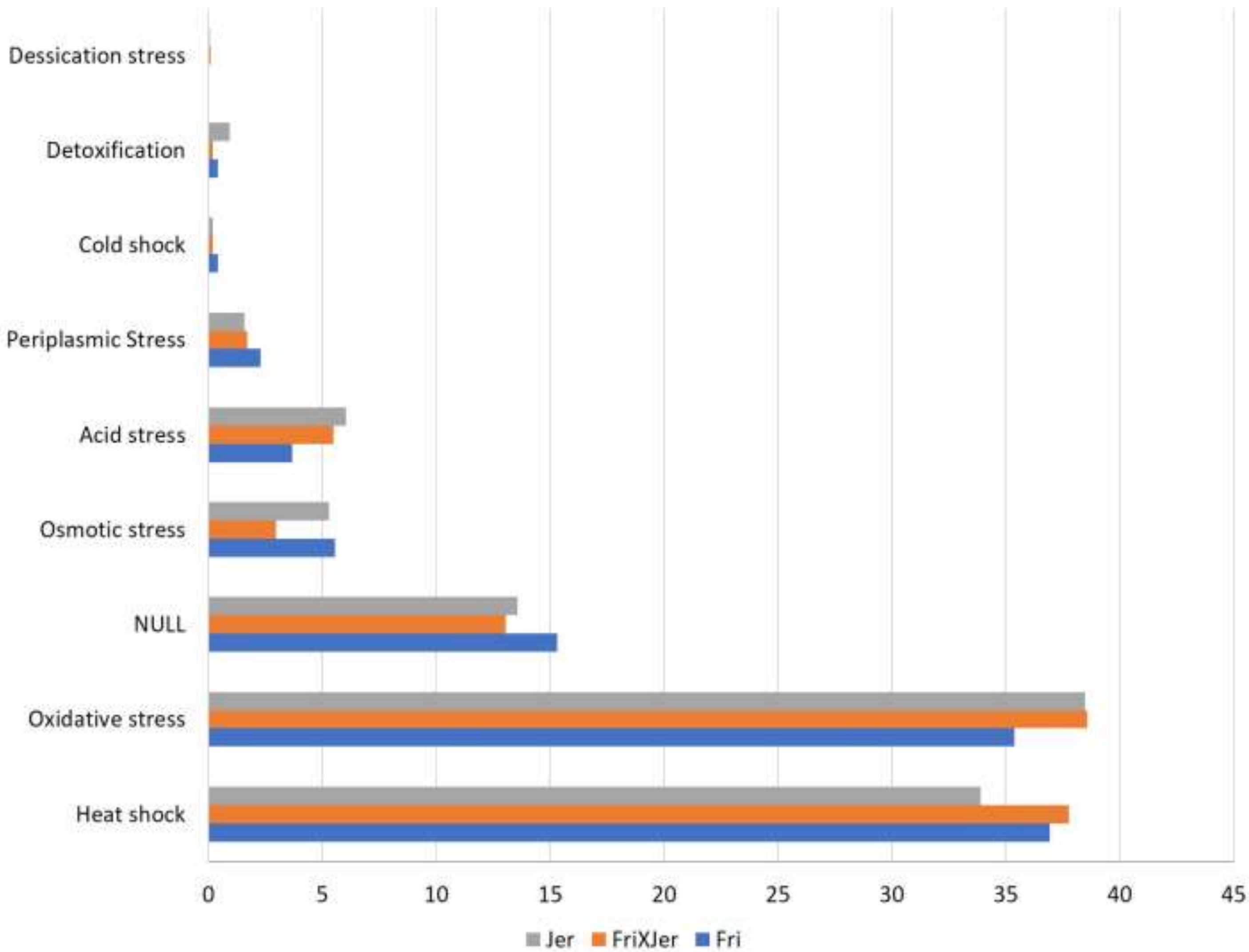

Figure 4. SEED subsystem composition of stress response genes in the three cattle genotypes. Fri = Friesian, Jer = Jersey and FriXJer = Friesian X Jersey cross.

Worth noting were the uniquely high proportions of Bacteroidia and Alphaproteobacteria in r1t-like streptococcal phages and phage packaging machinery respectively within the Jersey genotype (Table 8).

DISCUSSION

This research shows that shotgun next generation sequencing can also be used to identify relayed genes from smallholder cattle rumen metagenomes for resistance to antibiotics and toxic compound, phages and prophages and stress response. The shotgun technique outlined for deriving rumen microbiome profiles enables comparison of samples based on the entire population. Sample analysis on MG-RAST identified bacteria was predominant, followed by eukaryotes and viruses as the domains in our samples. This finding was in agreement with a previous study on cattle rumen microbiota (Pitta et al., 2016). At the phylum level, the sequences distribution indicates that the five most predominant clusters belonged to Bacteroidetes, Firmicutes, Proteobacteria, Actinobacteria and Fibrobacteres in all the cattle genotypes. This result was in congruent with findings from an earlier research by Jose et al. (2017a, b) who assesses the rumen microbial and carbohydrate-active enzymes profile in Indian crossbred cattle.

Similar to previous studies by Kalyuzhnaya et al. (2008) in cattle rumen and Singh et al. (2012) in buffalo rumen metagenome, the Level 1 subsystem classification showed that the largest percentage of gene fragments allocated to known features in all samples was correlated with clustering subsystems, carbohydrates and protein metabolisms. Genes in the clustering-based subsystems

Table 5. Abundance of stress response genes from small holder rumen metagenomes (%).

Stress response	Fri	FriXJer	Jer	P value
Heat shock dnaK gene cluster extended	**36.941**	**37.77**	**33.967**	0.489
Rubrerythrin	**9.794**	**9.263**	**10.512**	0.447
Regulation of Oxidative Stress Response	**8.283**	**9.892**	**9.264**	0.402
SigmaB stress response regulation	**7.316**	**7.014**	**5.651**	0.069
Oxidative stress	**6.348**	**6.385**	**6.241**	0.751
Redox-dependent regulation of nucleus processes	4.655	6.924	4.993	0.072
Hfl operon	4.111	4.406	4.862	0.41
Glutathione: Redox cycle	3.809	3.237	4.796	0.668
Acid resistance mechanisms	3.628	5.306	5.913	0.375
Carbon Starvation	2.479	1.169	2.169	0.078
Periplasmic Stress Response	2.297	1.709	1.577	0.667
Osmoregulation	2.237	1.439	2.169	0.547
Synthesis of osmoregulated periplasmic glucans	2.056	0.989	1.84	0.31
Protection from Reactive Oxygen Species	1.33	1.619	1.774	0.635
Choline and Betaine Uptake and Betaine Biosynthesis	1.209	0.36	1.183	0.307
Dimethylarginine metabolism	0.726	0.18	0.198	0.305
Bacterial hemoglobins	0.605	0.27	0.46	0.661
Glutathione: Non-redox reactions	0.484	0.45	0.263	0.786
Cold shock, CspA family of proteins	0.423	0.18	0.198	0.52
Glutathione: Biosynthesis and gamma-glutamyl cycle	0.423	0.45	0.657	0.467
Uptake of selenate and selenite	0.363	0.18	0.657	0.122
CoA disulfide thiol-disulfide redox system	0.121	0.27	0	0.636
Glutamate transporter involved in acid tolerance in Streptococcus	0.06	0.18	0.131	0.901
Glutathione-dependent pathway of formaldehyde detoxification	0.06	0	0.263	0.528
Phage shock protein (psp) operon	0.06	0	0	0.465
Ectoine biosynthesis and regulation	0.06	0.18	0.131	0.762
Glutaredoxins	0.06	0.09	0	0.649
Glutathione analogs: mycothiol	0.06	0	0	0.465
O-antigen capsule important for environmental persistence	0	0.09	0.066	0.637
Glutathionylspermidine and Trypanothione	0	0	0.066	0.465

Bold values indicate the predominance.

Table 6. Bacteria responsible for dnaK genes in the three cattle genotypes. Fri = Friesian, Jer = Jersey and FriXJer = Friesian X Jersey cross. Percent of dnaK genes within each metagenome that are assigned to the listed taxa.

Phylum	Class	Fri	FriXJer	Jer
Acidobacteria	Solibacteres	0.543	0.863	0.905
Actinobacteria	Actinobacteria (class)	3.96	1.393	2.136
Aquificae	Aquificae (class)	0	0.464	0
Bacteroidetes	Bacteroidia	31.665	31.152	27.896
	Cytophagia	2.409	2.808	2.383
	Flavobacteria	4.74	4.29	4.109
	Sphingobacteria	1.866	1.327	1.705
Chlamydiae	Chlamydiae (class)	0.352	0.309	0.431
Chlorobi	Chlorobia	1.659	0.773	1.952
Chloroflexi	Chloroflexi (class)	1.643	0.773	1.478
	Dehalococcoidetes	0.527	0	0.431
Cyanobacteria	Gloeobacteria	0	0.619	0.658
Deinococcus-Thermus	Deinococci	0.368	0	0.842

Table 6. Contd.

Dictyoglomi	Dictyoglomia	0.382	0	0.205
Elusimicrobia	Elusimicrobia (class)	1.484	1.172	2.568
Firmicutes	Bacilli	9.573	9.484	11.564
	Clostridia	14.084	15.565	17.278
	Erysipelotrichi	1.643	0.773	0.658
	Negativicutes	0.191	0	0.431
Fusobacteria	Fusobacteria (class)	1.452	2.344	0.658
Planctomycetes	Planctomycetacia	0.382	0.309	0
Proteobacteria	Alphaproteobacteria	7.179	5.218	5.63
	Betaproteobacteria	0.718	3.206	1.11
	Deltaproteobacteria	1.101	3.515	3.204
	Epsilonproteobacteria	1.147	1.172	0.615
	Gammaproteobacteria	7.851	8.977	7.54
Spirochaetes	Spirochaetes (class)	1.437	0.928	1.683
Synergistetes	Synergistia	0.191	0.155	0.205
Tenericutes	Mollicutes	0.895	1.791	1.274
Thermotogae	Thermotogae (class)	0.368	0.464	0.452
Verrucomicrobia	Opitutae	0.191	0	0
	Verrucomicrobiae	0	0.155	0
	Total (%)	**100**	**100**	**100**

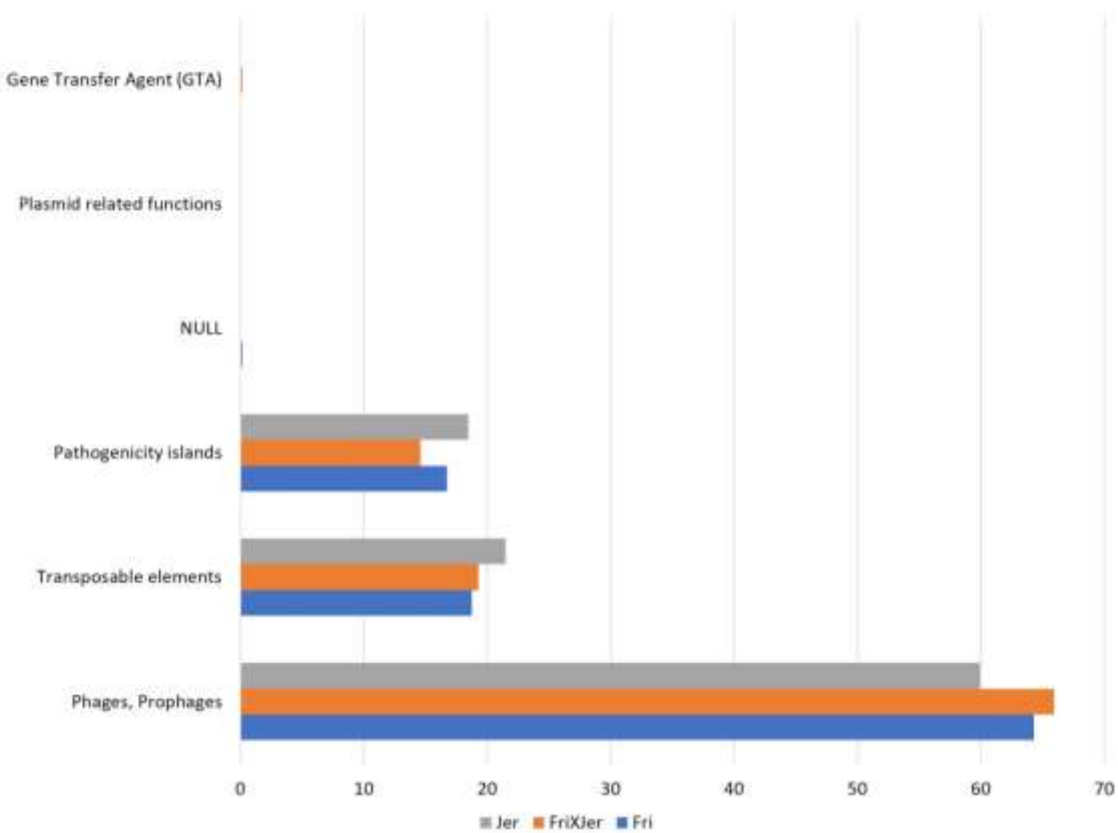

Figure 5. SEED subsystem composition of mobilome in the three cattle genotypes. Fri = Friesian, Jer = Jersey and FriXJer = Friesian X Jersey cross.

Table 7. Abundance of phages and prophages genes from smallholder rumen metagenomes (%).

Phages and prophages	Fri	Fri×Jer	Jer	P value
r1t-like streptococcal phages	32.512	33.8	28.755	0.111
Phage integration and excision	21.182	19.483	25.322	0.228
Phage replication	13.465	14.532	16.202	0.701
Phage packaging machinery	8.539	9.365	4.614	0.283
Phage regulation of gene expression	3.366	5.705	5.365	0.225
Staphylococcal phi-Mu50B-like prophages	4.598	3.445	3.648	0.35
Phage DNA synthesis	3.12	1.938	3.004	0.332
Phage introns	3.12	2.153	2.468	0.631
Phage entry and exit	2.956	3.66	2.79	0.023
Phage capsid proteins	2.545	1.507	1.824	0.412
Phage tail proteins 2	1.888	1.507	2.146	0.565
Phage tail fiber proteins	1.806	1.399	1.931	0.79
Listeria phi-A118-like prophages	0.411	0.215	0.429	0.981
Prophage lysogenic conversion modules	0.411	0.538	0.322	0.906
T7-like phage core proteins	0.082	0.215	0.215	0.609
Phage Dual Exonuclease Exclusion	0	0.431	0.215	0.506
Phage nin genes - N-independent survival	0	0.108	0.751	0.031

group are regularly observed together in various species for which particular features are not yet known (Durso et al., 2011). As observed in Figure 2, significant proportions of genes were assigned to the mobilome, resistome and stress genes in the SEED Subsystem Level 2 subcategory. Observation of antimicrobial resistance genes in this study serves to reinforce the theory proposed by Auffret et al. (2017), that the presence of AMR genes is largely linked to microbial stress response to dietary changes, since the animals in the study were reared in a farm with proper use of antimicrobials. Even more important, were the high correlations observed between these three functional groups (mobilome, resistome and stress genes). This result supports the hypothesis suggested by Reddy et al. (2014), which indicates that a complex network exists in the study of antibiotic resistance and stress genes, between bacterial pathogens and environmental reservoirs.

Specifically, within the RATC, the most frequently occurring functional groups were fluoroquinolone resistance genes, multidrug resistance efflux pumps and Methicillin resistance in Staphylococci. Similar observations were reported by Durso et al. (2012) in cattle feces microbiome within agricultural and non-agricultural metagenomes and by Cardoso et al. (2012) in snail metagenome. The finding of RATC genes in these cattle that had been reared in a farm with judicious use of antimicrobials further affirmed previous studies that suggested antibiotic resistance genes and antibiotic resistance occurs as an ancient phenomenon in a variety of human-impacted and pristine habitats (Kim and Bae, 2011; Reyes et al., 2010). Seed subsystem composition of phages indicated the predominance of pathogenicity islands that were majorly from phages replication, phage packaging and *rlt* streptococcal phage genes. This finding indicates that the expression of phage encoding genes in cattle rumen were a reflection of the induction of prophages in rumen bacteria. Genes associated with integrases and islands of pathogenicity were also identified similar to a previous research by Hacker and Kaper (2000). Genes involved in adapting to stress reactions were present in all metagenomes. These comprised of the genes encoding for Chaperone protein (DnaK), Chaperone protein (DnaJ) and nucleoside 5-triphosphate RdgB. These genes play an important role in adaptations to psychrophilic lifestyles (Rodrigues and Tiedje, 2008). Contrary to previous studies, in this study, the cattle rumen metagenomes had an enhanced representation of the Sigma B gene, a general stress regulon which induces a wide range of genes in response to various stressful conditions including heat, acid, salt and starvation (Höper et al., 2005). Matches allocated to the genes of more-constituent proteins connected with cold adaptation chaperones DnaK and DnaJ were also of significant interest due to their high abundance in the rumen microbial communities. These genes have been shown to be induced in bacteria when exposed to cold temperatures (Cavicchioli, 2006). We theorize that the presence of these genes could be due to the cold climate in which the animals were reared.

The findings of this study also showed that Clostridia and Bacilli were the predominant bacterial taxa actively playing a part in the resistome, mobilome and stress reactions in the current research. This was in agreement with previous studies on the Indian Buffalo rumen (Reddy et al., 2014). In both the SEED Subsystem Levels 1 and

Table 8. Bacteria responsible for **A**. r1t- like streptococcal phages, **B**. Phage integration and excision C. Phage packaging machinery and **D**. phage replication in the three cattle genotypes. Fri = Friesian, Jer = Jersey and FriXJer = Friesian X Jersey cross.

Phylum	Class	Fri	FriXJer	Jer
A. r1t-like streptococcal phages				
Actinobacteria	Actinobacteria (class)	2.428	0	0
Aquificae	Aquificae (class)	0	0.37	1.488
Bacteroidetes	Bacteroidia	7.594	6.346	16.883
	Cytophagia	0	0.37	0
	Flavobacteria	5.472	3.759	3.686
	Sphingobacteria	1.432	2.279	0
Chlamydiae	Chlamydiae (class)	0	0.37	0
Chlorobi	Chlorobia	0.871	0.37	0
Chloroflexi	Dehalococcoidetes	10.886	11.645	8.862
Dictyoglomi	Dictyoglomia	0.437	0	0
Elusimicrobia	Elusimicrobia (class)	0	0.37	0
Firmicutes	Bacilli	39.147	43.991	34.409
	Clostridia	24.264	21.502	19.083
Fusobacteria	Fusobacteria (class)	0	0	0.776
Proteobacteria	Alphaproteobacteria	2.303	4.498	6.726
	Betaproteobacteria	0	0.37	2.2
	Deltaproteobacteria	1.118	0.739	0.776
	Gammaproteobacteria	3.611	3.02	5.11
Spirochaetes	Spirochaetes (class)	0.437	0	0
	Total (%)	**100**	**100**	**100**
B. Phage integration and excision				
Bacteroidetes	Bacteroidia	57.612	58.969	65.184
	Cytophagia	4.834	7.109	1.044
	Flavobacteria	7.018	7.491	8.048
	Sphingobacteria	0.811	0	0.404
Chlorobi	Chlorobia	0.561	0	0
Deferribacteres	Deferribacteres (class)	0	0	0.404
Firmicutes	Bacilli	9.67	9.661	4.748
	Clostridia	7.018	9.78	7.474
Fusobacteria	Fusobacteria (class)	2.495	0	0.64
Proteobacteria	Alphaproteobacteria	3.056	1.145	6.093
	Betaproteobacteria	0.967	0.763	0.64
	Deltaproteobacteria	1.684	0	1.852
	Epsilonproteobacteria	0	0.382	0
	Gammaproteobacteria	1.372	0.382	1.213
Spirochaetes	Spirochaetes (class)	1.778	0.763	0
Tenericutes	Mollicutes	0.561	0	0
Thermotogae	Thermotogae (class)	0.561	3.554	2.256
	Total (%)	**100**	**100**	**100**
C. Phage packing machinery				
Actinobacteria	Actinobacteria (class)	1.471	0	0
Bacteroidetes	Bacteroidia	2.941	4.167	4.545
Deinococcus-Thermus	Deinococci	2.941	0	0
Firmicutes	Bacilli	45.588	45.833	18.182

Table 8. Contd.

	Clostridia	25	27.083	31.818
Fusobacteria	Fusobacteria (class)	4.412	4.167	4.545
Proteobacteria	Alphaproteobacteria	4.412	6.25	22.727
	Betaproteobacteria	0	2.083	0
	Epsilonproteobacteria	1.474	0	9.091
	Gammaproteobacteria	11.765	10.417	9.091
	Total (%)	**100**	**100**	**100**
D. Phage replication				
Actinobacteria	Actinobacteria (class)	2.756	2.804	2.294
Aquificae	Aquificae (class)	0.672	0	0
Bacteroidetes	Bacteroidia	43.524	32.325	42.365
Bacteroidetes	Cytophagia	0	0	0.617
Bacteroidetes	Flavobacteria	6.114	7.123	5.474
Bacteroidetes	Sphingobacteria	0.689	1.682	0
Chlamydiae	Chlamydiae (class)	0	1.814	0
Chlorobi	Chlorobia	0	0.56	0
Chloroflexi	Dehalococcoidetes	4.082	8.114	9.797
Deinococcus-Thermus	Deinococci	0	1.814	0
Elusimicrobia	Elusimicrobia (class)	1.378	2.374	1.678
Firmicutes	Bacilli	19.738	16.358	9.621
Firmicutes	Clostridia	14.244	18.041	16.762
Proteobacteria	Alphaproteobacteria	1.36	1.122	1.678
Proteobacteria	Betaproteobacteria	1.36	0.56	0
Proteobacteria	Deltaproteobacteria	0.689	0.56	2.12
Proteobacteria	Epsilonproteobacteria	1.378	0	0
Proteobacteria	Gammaproteobacteria	2.015	4.188	6.535
Tenericutes	Mollicutes	0	0.56	1.06
	Total (%)	**100**	**100**	**100**

Percent of genes within each metagenome that are assigned to the listed taxa.

2, the resistance genes, phage and prophage genes and stress responses had statistically no significance, thus giving an indication that the genotype of animals did not affect these microbial functions.

This pilot study elucidates the resistome, mobilome and stress responses in Friesian, Jersey and FriesianXJersey crossbreed cows reared in the tropics. Additionally, the study demonstrates that despite the judicious use of antimicrobials, the rumen microbiota in the study animals had a vast assemblage of antimicrobial resistance genes. The study also showed that there exists a correlation between stress genes, antimicrobial resistance genes and mobile genes. Moreover, we found no support for the hypothesis that the resistome, mobilome and stress responses were correlated with the animal breed with minimal differences observed in gene abundance between the three cattle genotypes. However, validating these results using more cows per breed and increasing the sampling repetitions in future studies could undoubtedly augment our understanding of the disparity between individual cows and breeds. In addition, comparing the results with farms where there is indiscriminate use of antimicrobials could present better insights into these microbial profiles.

Availability of data and materials

The datasets generated and/or analysed during the current study are available in the https://www.mg-rast.org/linkin.cgi?project=mgp79210, using the following MG-RAST IDs; mgm4736079.3, mgm4752069.3, mgm4748083.3, mgm4752189.3, mgm4751799.3, mgm4735643.3

Authors' contributions

FK, RB, CG, EM, FM designed the research project and helped prepare the manuscript. FK, FM conducted the bioinformatics and statistical analysis. FK, EM, and FM performed the laboratory analyses. All authors read and approved the final manuscript.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Laboratory evaluation, purpose of production and utilization of cattle manure in *enset* (*Ensete ventricosum*) based mixed production systems of Gurage Zone, Southern Ethiopia

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The study was conducted in four districts of Gurage zone in Sothern Ethiopia to determine the N, P, K, organic carbon and nitrogen of cattle manure. Seventy two households, 36 from highland and 36 from midaltitude were randomly selected from a total of 360 sample households selected for the study. Around 88.05% of farmers in the study areas were keeping cattle for high demand of manure to fertilize enset fields and for milk production. There were no practices of using cattle dung as fuel or dung cake for sale. The sampled manure had nitrogen content of 2.68% and C/N ratio of 11:1 in highland and 2.24% with C/N ratio of 12:1 in mid-altitude. The organic matter obtained from manure in highland and midaltitude, respectively, were 51.89 and 44.82%. The gram of N, P, K, kg^{-1} DM of manure, respectively, were 26.8, 16.5 and 1.6 for highland and 22.4, 12.6 and1.2 for midaltitude. To realize production sustainability of *enset* system, cattle manure was found to be of paramount importance. Therefore, appropriate interventions in cattle production and forage development are the prime necessity to realize sustainability in enset production and households' food security.

Key words: Cattle manure, Gurage Zone NPK, organic carbon, organic matter.

INTRODUCTION

Cattle are an important component of nearly all farming systems in Ethiopia and provide draught power, milk, meat, manure, hides and serve as a capital asset against risk (Ehui et al., 2002). Manure production is also considered important by most crop/livestock and agro-pastoralist farmers, but as secondary rather than a primary purpose (Alemayehu, 2004; Mekonnen and Köhlin, 2008). Manure is a complex material that contains valuable nutrients and used as a fertilizer for centuries and contains several essential plant nutrients which contributes to increased crop yields when properly applied to soils. Thus, dairy and other livestock producers can use manure as a valuable source of fertilizer nutrients for crops (Jodie et al., 1914).

Southern Ethiopia in general and Gurage zone in particular is characterized by production of enset (Ensete

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ventricosum) which needs cattle manure for its sustainable production and productivity. As a perennial and maturing at around 5 to 10 years, *enset* acts as a food store (security food crop) which can be used at any time of the year. It is relatively resistant to drought, heavy rain, and seasonal flooding which ordinarily devastate other food crops, especially cereals (Brandt et al, 1997; Million et al., 2003; Tesfaye, 2005).

In Gurage zone, a prudent interaction is there between *enset* and cattle production particularly in the altitudes where *enset* serves as a source of fodder for cattle and cattle provide manure to fertilize *enset* fields. In the absence of cattle manure in this system, the sustainability of *enset* production will definitely and negatively be affected. Hence, identifying the existing situations in relation to the purpose of cattle manure production, utilization and its major chemical composition in the area is crucial for further interventions.

Therefore, this study was conducted in the highland and midaltitude agroecologies of Gurage zone with the following specific objectives:

(1) To evaluate the major chemical composition of cattle manure in the laboratory.
(2) To assess the purpose of cattle manure production and utilization.

MATERIALS AND METHODS

Description of the study area

Based on data from Gurage zone Department of Finance and Economy Development (DoFED, 2015), the study area, Gurage zone, is found in the Southern Ethiopia. It is located between 37° 28' and 38° 38' longitude and 7° 28' and 8° 27' latitude, covering an area of about 5,932 km^2 (DoFED, 2015). Gurage zone has 13 Administrative districts with 412 Peasant Associations (PAs) and 2 town administrations (DoFED, 2015). The zone is bounded with Oromiya regional state in the north, northeast and northwest, Silti zone in the south east, Hadiya zone in the south and Yem special district in west directions. Wolkite, the capital of the zone, is 155 km away from Addis Ababa in the Addis-Jimma road (DoFED, 2015).

The estimated human population of the zone is 1,624,125 (51.4% women and 48.6% men) and 88.2% of the population are farmers entirely dependent on subsistent agriculture (DoFED, 2015; CSA, 2016b). Gurage zone is one of the most densely populated areas in the country with an average of 273.5 people/km^2 mainly concentrated in the agroecology of highland and midaltitude. Based on data from the Department of Agriculture and Natural Resource Development of Gurage zone (DANRD, 2016), the zone is found in altitudinal range of between 1600 and 3100 m above sea level (masl). The major crops grown in this area are *enset (E. ventricosum*), Barely (Hordeum vulgare), Field pea (*Pisum sativum*), Fava bean (*Phaseolus vulgaris*), *Teff* (*Eragrostis teff*), Maize (Zia mays) and Khat (*Catha edulis*) (DANRD, 2016).

The average annual temperature of Gurage zone is about 18°C. The current land use pattern of the zone, is 398,887 ha of land for crop production, 92,421 ha for grazing, 42,933 ha for forest, 17,168 ha degraded land and 41,791 ha of land for other social services giving institutions. Livestock population of 3,611,159 are found in the zone, constituting 1,678,455 cattle, 616,900 sheep, 260,420 goats, 820,269 chickens, 128,532 horses, 9,464 mules and 97,119 donkeys (DoFED, 2015; CSA, 2016a).

Sampling and sample size determination

Information on nature of PAs in relation to livestock population and *enset* (*E. ventricosum*) production was obtained from zonal and district departments of Agriculture and Natural Resource Development. Peasant associations were identified after having *enset* and livestock population data and a total of 8 PAs (2 PAs from each district/one highland and one midaltitude) were purposively selected based on cattle number, *enset* production and accessibility. Households sample size was determined using (Cochran, 1909; Thrustfield, 2013) sample size determination formula:

$$n = Z^2 \times P(1-p)/e^2; \text{ n adjusted} = n/[1+ ((n-1)/N)]$$

where n = sample size in population, Z-score = 1.96 for confidence level 95%, N = total HHs of 4 study districts, P = proportion of population score of 1= 0.5, 1-p = 0.5 and e = standard error of proportion = α = 0.05. A total of 360 HHs from 8 PAs (45 HHs from each PA) were selected for the study. The selected PAs from highland and midaltitude in each district, respectively, were Shamene and Shehremo from Ezia district; Achene and Wukiye from Muhir and Aklil districts; Moche and Yeferezye from Cheha district as well as Agata and Kochira from Enemor and Aner districts.

Design of the study

To assess the purpose of cattle manure production and utilization, pre-tested survey questionnaires were used to collect data from 360 sample households of the study. Before commencement of the survey, one-day training was organized for enumerators on how to administer the questionnaire. Interview was done by researcher together with the enumerators and Development Agents (DAs).

Methods of data collection

The amount of cattle manure produced and the percentage of manure utilized by the HHs for different purposes in the study area was assessed in each study PA during the survey at household's home using survey questionnaire along with personal observation. The data on the use of manure in relation to different crops were also collected through interviewing the household. To obtain additional information, group discussions were made at zonal and each district level to clarify issues not well addressed through survey and to validate some information collected from individual interview. A total of 5 group discussions comprising 44 individuals, 9 from each district (5 farmers, 2 experts and 2 DAs) and 8 experts from zonal office (6 from livestock and 2 from crop agriculture) participated in the group discussion.

Sampling and drying process of cattle manure

Cattle manure used for the laboratory evaluation was obtained from 72 randomly selected HHs (36 from highland PAs of above 2200 masl) and 36 from midaltitude PAs of between 1800 and 2200 masl from a total of 360 sample households selected for the study. Fresh manure samples of 2 kg from overnight dropping were collected from each selected HH using plastic bag. The drying process was carried out by thinly spreading of cattle manure on polyethylene plastic sheet separately based on respective agroecology under shade. Cattle manure was turned and mixed up several times a day

Table 1. Objective of cattle rearing in the study areas of Gurage zone (%).

Study districts	Milk only	Manure only	Traction only	Milk and traction	Milk and manure
Ezia (n= 90)	0	0	0	15.6	84.4
Muhir and Aklil (n= 90)	0	0	0	4.4	95.6
Cheha (n= 90)	0	0	0	8.9	91.1
Enemor & Aner (n= 90)	0	0	0	18.9	81.1
Total (N=360)	0	0	0	11.95	88.05

N = Total sample households of the study, n = sample households per district.

to break large particles formed and to ensure uniform drying. At the end of the drying process, representative samples of air dried manure from each agroecology were taken. The air-dried cattle manure was ground in a laboratory to pass 2 mm mesh (Abbasi et al., 2007) at Wolkite regional soil analysis laboratory and packed in an airtight clean plastic bag and stored until required for analysis. The manure samples were analyzed for N, P, K and Organic carbon (Nelson and Summers, 1982)

Chemical analysis of cattle manure samples

Some selected properties of manure such as organic carbon, total nitrogen, phosphorus and potassium were determined following standard procedures (Nelson and Summers, 1982). The percent organic matter in cattle manure was calculated by multiplying the percent organic carbon by an empirical factor of 1.724 or 100/58, following the standard practice that organic matter conventionally assumed to contain 100/58 of % organic carbon (Nelson and Summers, 1982).

% Organic matter = 1.724 × % Organic carbon

The ratio of carbon to nitrogen content (C: N) of manure was calculated as percentage of carbon in the manure divided by the percentage of nitrogen obtained in cattle manure (Nelson and Summers, 1982).

Carbon to Nitrogen Ratio (C: N) = % Carbon / % Nitrogen

Organic carbon on the other hand was determined using the Walkley-Black rapid titration method (Nelson and Summers, 1982).

%Organic carbon = N × (V1-V2/S) × 0.39 × mcf

where N = normality of ferrous sulfate solution (from blank titration), V1= ml of ferrous sulfate solution used for blank, V2 = ml of ferrous sulfate solution used for sample, S = weight of air-dried manure sample in gram, $0.39 = 3\times10^{-3}\times100\times1.3$ (3 = equivalent weight of carbon), mcf = moisture correction factor.

Total nitrogen was analyzed by Kjeldahl method (Yerima, 1992). Accordingly, the phosphorous (P) and potash (K) contents of manure were determined by atomic absorption spectrophotometer (Perkin, 1982).

Methods of data analysis

The collected data were analyzed in such a way that they met research objectives and answer research questions. The study involved both qualitative and quantitative data analysis techniques. Information generated from sample households interview, group discussion and personal observation were discussed and narrated qualitatively. Statistical package for social sciences, version 20 (SPSS, 2012) was used for analysis of collected data after checking, correcting and coding. Descriptive statistics such as table, percentage, mean and standard error was used to present the results.

RESULTS AND DISCUSSION

Objectives of cattle rearing

According to the responses of households (Table 1), the primary objective of rearing cattle by the households was necessity for high demand of manure to fertilize crop land particularly of *enset* fields and milk production to supplement *enset* product (*kocho*) which is low in protein. About 84.4, 95.6, 91.1 and 81.1% of the households in the study districts of Ezia, Muhir and Aklil, Cheha and Enemor and Aner, respectively, were keeping cattle primarily for the production of milk and manure. Some respondents of around 18.9, 15.6, 8.9 and 4.4%, respectively, from Enemor and Aner, Ezia, Cheha and Muhir and Aklil districts held cattle primarily for milk production and traction.

In general, around 88.05% of the livestock owners in these areas engaged in the production of cattle aiming majorly on production of milk to support *enset* based food to lead healthy life and manure to fertilize crop garden which is in agreement with the result of Beriso et al. (2015) from Aleta Chukko district of Southern Ethiopia, who reported cattle keeping was important component of the mixed-farming system that cattle provide, milk and fertilizer (manure). Similar result was also reported by Snijders et al. (2009) who indicated that smallholder farmers in Central Kenya, for example, highly value dairy cows for the production of manure, in addition to their production of milk.

Purpose of cattle manure production

Cattle manure in *enset* production system of Gurage area plays a critical role in maintaining soil fertility and agricultural sustainability. In this low input farming systems the primary purpose of cattle production was to produce manure to fertilize *enset* (Table 2). At the same

Table 2. Purpose of cattle manure production in the study areas of Gurage zone.

Study district	Uses of cattle manure (%)		Manure used for different crops (%)		
	Used as fertilizer	Used as fuel	*Enset* crop	Vegetables & root crops	Khat, coffee &fruits
Ezia (n = 90)	100	0.0	92.0	3.0	5.0
Muhir & Aklil (n = 90)	100	0.0	90.0	4.0	6.0
Cheha (n = 90)	100	0.0	93.0	2.0	5.0
Enemor & Aner (n = 90)	100	0.0	90.0	3.0	7.0
Over all (N = 360)	100	0.0	91.3	3.0	5.7

N = Total sample households of the study, n = sample households per district.

time, 100% of households (Table 2) and focus group discussants (FGD) who participated on the study replied that 100% of cattle manure produced were used for fertilizing the crop lands. From the total manure produced in the study areas, 91.3% was used for enset farms fertilization while 3.0 and 5.7%, respectively, was used for the fertilization of vegetable crops and for khat, coffee and fruits. The participants of the study also confirmed that with no doubt, in the absence of cattle manure in this system, *enset* production could be deprived of sustainability because cattle manure is the principal source of organic matter and nutrient input of *enset* plant. Household participants also realized the absence of any tendency of utilizing farm yard manure for household energy requirement either for cooking or heating.

As indicated in Table 2, households involved in the study also revealed that the only purpose of cattle manure production was to be used as fertilizer and in the study areas of Gurage zone there were no practices of using cattle dung as fuel or making of dung cake for sale which is in line with the report of Muhereza et al. (2014), who reported that 100% of farmers in central Uganda utilized cattle manure on their crop garden and there was no practices of making dung cake for sale or using dung as fuel. In the same way, Snijders et al. (2009) in their report revealed that manure is an important source of nutrients for many smallholder farmers in East Africa, with cattle manure being the dominant type. The result of the current study in relation to the purpose of cattle manure production, however, does not agreed with report of Alemayehu (2004) who stated that manure production is considered important by most crop/livestock and agro-pastoralist farmers in Ethiopia, but as secondary rather than a primary purpose

The result of this work, on the other hand, was not in agreement with report made by Mekonnen and Köhlin (2008), who indicated that from the highlands of Ethiopia where sedentary agriculture is practiced, most of the manure produced is used as fuel, especially in the central and northern part of Ethiopia and only a very small fraction is used for manuring the soil and its use as manure is generally limited to small area of land around the homestead or nearby farms. The same authors revealed also that each farm household in the central and northern part of the country was using dung for his household energy requirement essential for cooking and heating.

Lupwayi et al. (1999) also reported that manure collected from farms of Ethiopian highlands in Deneba area had significantly greater contents of N, P, K, but due to scarcity of fuel wood, farmers in Deneba were using manure for fuel for domestic cooking and heating instead of applying it to the soil which is not in agreement with the report of the current study. The result of the current study also disagreed with the result reported by Yilma (2001), from Sidama zone of Southern Ethiopia indicated, around 20% of livestock dung was used as a source of fuel and the rest (80%) was used for farm yard manure.

Households' potential of producing cattle manure

From a total of 360 household participants of both agroecologies, about 77.25% reported their inability of having potential to produce enough amount of cattle manure to fertilize their crop garden (Table 3) due to an increase in human population that enhanced fragmentation of land distributed to individual farmer. The respondent households also revealed that the fragmented land size allotted to individual household, worsened the ability of household to produce forage enough to feed his cattle. Reduction in household's forage producing capacity correspondingly associated with reduction with the number of cattle and minimizing total amount of manure produced.

The production of low amount of livestock feed also accounted for the reduction of manure produced per animal. The result of the current study agreed with the result reported from central Uganda by Muhereza et al. (2014) who reported that farmers fertilized portions of the farm on a rotational basis according to perceived soil nutrient deficiency. The same authors also indicated that cattle manure was not adequate to fertilize the whole farm in a single cropping season as the result of inadequacy of manure due to small herd size, lack of supplementary feeding and inadequate fodder production due to limited land available. The result of the current study also corresponds with the report of Maryo et al.

Table 3. Households' potential on producing cattle manure in the study areas of Gurage zone.

Manure production adequacy (%)	Study districts (n = 90)				Agroecologies (n = 180)		Over all (N= 360)
	Ezia	Muhir & Aklil	Cheha	Enemor & Aner	Highland	Midaltitude	
Yes	26.7	21.1	24.4	18.9	22.2	23.3	22.8
No	73.3	78.9	75.6	81.1	77.8	76.7	77.2

N = Total sample households of the study, n = sample households per district and agroecology.

Table 4. Perception of HHs on significance of cattle manure in the study areas of Gurage zone.

Significance of cattle manure (%)	Study districts (n = 90)				Overall (N=360)
	Ezia	Muhir & Aklil	Cheha	Enemor & Aner	
Sustainable production and productivity	100	100	100	100	100
Low costly than inorganic fertilizer	96.7	87.8	94.4	98.9	94.4
Sustain soil fertility and reduce erosion	95.6	92.2	98.9	100	96.7
Easily available than inorganic fertilizer	94.4	94.4	87.8	93.3	92..5

N = Total sample households of the study, n = sample households per district.

(2014) who stated the existence of strong interaction between *enset* and cattle. *Enset* provides feed to the cattle and cattle provide manure to fertilize *enset* fields which enhances the production and productivity. In the absence of cattle manure, the sustainability of *enset* production can certainly and negatively be affected.

Perception of households on significance of cattle manure

Households and group discussants (100%) who participated in the interview perceived that without the application of cattle manure, the sustainability of crops particularly *enset* production could be affected negatively. The result of current study agreed with the report of Brandt et al. (1997) and Maryo et al. (2014) who indicated that different factors contribute to the progressive downward spiral in cattle production sector of the rural economy. This decline in cattle size affects the level of manure production and decreases in cattle manure causes reductions in the long-term sustainability of *enset* production that could also have an impact on human nutrition.

In *enset* farming system of the study area, cattle manure is the major source of organic matter, nutrient input and is critically important for productivity of the system. This is in agreement with the report of Snijders et al. (2009) who stated that manure is an important source of nutrients for many smallholder farmers in East Africa, with cattle manure being the dominant type. Based on the result of the current study as indicated in Table 4, 100% of the households reported that sustainability of production and productivity of *enset* cannot be achieved without the application of cattle manure. On the other hand, 96.7% of respondent households in both agroecologies, indicated the significant role of cattle manure in keeping sustainability of soil fertility and reducing soil erosion by improving the organic matter content thereby promoting the percolation and infiltration of run off.

Similarly, the members of group discussion of the study also emphasized that cattle manure is the principal source of organic matter and nutrients for crop production, particularly important for productivity of *enset* garden. This is in agreement with the report of Tadesse (2013), who reported that manure plays a vital role in improving crop yields and allowing sustainable productivity and has ability of changing soil microclimate condition and restoration of ecological balance. As indicated in Table 4, due to manure's ease of availability and low cost when compared with inorganic fertilizer, cattle manure was chosen, respectively by 92.5 and 94.4% households of both agroecologies. This corresponded with the report of Muhereza et al. (2014) who reported that the major benefits obtained from the use of cattle manure included increased crop yields, disease reduction, easily availability of cattle manure and low cost of purchasing the manure.

Correspondingly, the household participants and members of group discussion of the study also reported that the use of inorganic fertilizers was impossible to be used for *enset* crop production due to its high cost and limited availability. The result of the current study also corresponded with the report of Risse et al. (2006) who indicated that the use of inorganic fertilizers is limited for *enset* crop because of its high cost and limited availability. Thus, cattle manure is a locally available low

Table 5. Cattle manure chemical composition in the study area of Gurage zone.

Agroecology	Chemical composition of cattle manure							
	Nitrogen		Phosphorus		Potassium		OC	OM
	%	(g/kg DM)	%	(g/kg DM)	%	(g/kg DM)	%	%
Highland	2.68	26.8	1.65	16.5	0.16	1.6	30.1	51.89
Midaltitude	2.24	22.4	1.26	12.6	0.12	1.2	26	44.82
Overall mean	2.46	24.6	1.45	14.55	0.14	1.4	28.05	48.35

OC = Organic carbon, OM = organic matter, DM = dry matter, Moisture content of cattle manure = 25.3% for midaltitude and 29.4% for highland. Source: Own sample collected (2017/2018).

cost substitute for the majority of resource poor farmers. Apart from its low cost and local availability, cattle manure is highly valued by farmers because of its multiple roles and long-term benefits. Similar conclusion was made by Maryo et al. (2014) that decline in cattle number will call for decline in cattle manure production which cause reductions in the long-term sustainability of *enset* production and productivity. The cycle of increasing impoverishment on the cattle component in this mixed crop/livestock system is a serious cause for concern.

Chemical composition of cattle manure

The result of chemical composition of manure collected from sample households in the study areas of Gurage zone is shown in Table 5. The manure samples used in the study had a total nitrogen content of 2.68% in highland and 2.24% in midaltitude agroecologies with overall average of 2.46% on dry weight basis. This result is in agreement with the report of Snijders et al. (2009) who indicated that the total nitrogen content of manure on a dry matter basis ranges from below 0.5 to over 4%.

At the same time, the carbon to nitrogen ratio of manure for both agroecologies was assessed and the C: N ratio of 11:1 for highland and 12:1 for midaltitude with an overall average C: N ratio of 11.5:1 which was calculated as percentage of carbon in the manure is divided by the percentage of nitrogen obtained in cattle manure (Nelson and Summers, 1982). The results obtained in the current study on the percentage of total nitrogen and carbon to nitrogen ratio of cattle manure is different from the result reported from southern Ethiopia by Ferew (2012) who found that the manure used in his study had total nitrogen of 1.89% on dry weight basis and a C: N ratio of 18:1. On the other hand, the moisture content of cattle manure in the current study at different agroecology was different and it was about 25.3% for midaltitude and 29.4% for highland agroecology.

The difference in total nitrogen, organic carbon, carbon to nitrogen ratio and moisture content among the study areas in particular and in the country in general could mainly be attributed to differences in environmental conditions of climate, soil, chemical content of the feeds consumed by cattle such as crop residues of different types, variation in legumes to grass composition, leaf and leaf midribs of *enset* available to cattle feeding. The aforementioned reasons attributed for differences in cattle manure chemical composition on different agroecologies are in agreement with the report of Lupwayi et al. (1999) who stated that manures collected from experimental stations contained significantly more N, P, K, than the manure rom smallholder farms, probably due to differences in the type and quality of available feed and other factors. Snijders et al. (2009) on their report also indicated the existence of large variation in nitrogen (N), phosphorus (P), potassium (K) and carbon (C) contents of cattle manures from Africa. The same authors also indicated that manure quality strongly varies, due to variation in feed supply and intake, ration quality, addition of organic material to excreta, losses during collection and storage and contamination with soil.

The composition of organic matter estimated in the manure samples analyzed in the laboratory in the current study was 51.89% for manure samples taken in highland and 44.82% for midaltitude agroecology which is significantly important to assure the sustainability of the system. Similarly, the main mineral nutrients that were incorporated in the samples of cattle manure were identified and the gram of nitrogen (N), phosphorus (P) and potassium (K) kg^{-1} DM of manure, respectively, were 26.8, 16.5 and 1.6 in the case of highland and 22.4, 12.6 and1.2 for midaltitude agroecology which are very important in the improvement of soil contents and thereby the production and productivity of the systems. The result of current study corresponded with the report of Risse et al. (2006) who stated cattle manure as an excellent source of plant nutrients such as nitrogen, phosphorus and potassium as well as the secondary nutrients that plants require.

The results of the current study in the contents of manure in highland is much higher than the contents of manure in midaltitude which could mainly be due to the availability of more legumes and other feed resources with better nutrients and mineral components in highland areas than the midaltitude. This report is in agreement with the report made by Snijders et al. (2009) who indicated that contents in farm yard manure particularly the nitrogen from temperate countries are often higher, probably due to higher protein contents in feed rations and more favorable collection and storage conditions, including lower temperatures that relatively reduces microbial activities.

The gram of nitrogen and phosphorus kg^{-1} DM of cattle manure reported in the current study is also much higher than the result of 18.9 g N and 6g P kg^{-1} DM of manure reported by Ferew (2012) and the average result of 18.3 g N, 4.5 g P kg^{-1} of cattle manure on dry matter basis by Lupwayi et al. (1999). However, the gram of potassium obtained kg^{-1} DM of manure samples in the current study is extremely lower than the reported value of 21.3 g potassium kg^{-1} DM of cattle manure by Lupwayi et al. (1999) which needs special attention in the future.

The organic matter in cattle manure in the current study is dependent on the percent organic carbon found in the cattle manure. It was indicated by the household respondents and group discussants of the study that the application of cattle manure increases the amount of organic matter and thereby determining physical and chemical nature of soil. The result of the current study corresponded with the report of Risse et al. (2006) who stated that the application of cattle manure increases the level of soil organic matter. Soil organic matter is known to affect a number of soil chemical properties such as the cation exchange capacity and the soil buffering capacity that enable manure treated soils to retain nutrients and other chemicals for longer periods of time.

Conclusion

The primary objective of rearing cattle in the study areas of Gurage zone of Ethiopia is for high demand of manure to fertilize crop land particularly *enset* fields and milk production to supplement *enset* product (*kocho*) which is low in protein in the nutrition of the farmers. Households in this *enset*-cattle based mixed production system have perceived the existence of strong linkage between cattle and *enset* production and considered cattle manure as basic source of soil nutrients (N,P,K) to be used by field crops. Cattle manure has the greatest value towards improving crop production; however, the households in the study areas are not in a position to produce manure sufficient to fertilize their crops. Reduction in grazing lands and cattle number per individual household, worsened household's ability to produce manure enough to fertilize crop garden. Therefore, appropriate interventions in cattle production and forage development are the prime necessity to realize sustainability in *enset* production and households' food security.

CONFLICT OF INTERESTS

The author has no declaration of any conflict of interests.

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Effect of garlic (*Allium sativum*) and onion (*Allium cepa* L.) extract chitosan nanoparticles on antioxidant enzymes activities and relative weight of visceral organs of rainbow rooster chicken

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Poultry meat is susceptible to oxidation but increased antioxidant enzyme increases its availability in muscle during processing and storage. In livestock, synthetic antioxidant has some side effects; plant polyphenols can enhance the level of antioxidant enzymes but they are in-active in the gut of chicken and therefore, nanotechnology can be of importance in augmenting the stability of polyphenols. In the study, seventy two rainbow rooster chickens were treated with nanoparticles- prepared from Chitosan with Aqueous Garlic and Onion (CHIAGO), chitosan with total phenol and ajoene rich extract (CHITPA), and chitosan solution (CHISOLN) of 5 to 10%, with 0.5 g and 1 g Fosbac (antibiotic) administered orally twice a week for a period of 8 weeks. One chicken from each of the group and a control group were sacrificed on weekly basis with the muscles and visceral organs removed for analysis. The weight of visceral organs, catalase (CAT) enzyme activities of thigh and breast were analyzed from 1st to 8th weeks and 2, 2-Diphenyl-1-picryl hydroxyl (DPPH) inhibition of thigh and breast muscles for 1st, 4th and 8th weeks. Relative weight of the heart, liver and spleen did not change when compared with the control (*p*>0.05) but it increased in the gizzard (*p*<0.05). Catalase increased in thigh and breast muscles (p>0.05) but without increase in the erythrocytes and liver (*p*>0.05). DPPH inhibition increased with CHIAGO 10%, CHITPA 10% in week 4 and week 8 in the breast and thigh muscle. Garlic and onion extract chitosan nanoparticles can act as natural antioxidant compound.

Key words: Chitosan, garlic and onion extract, visceral organ, meat, antioxidant enzyme activities.

INTRODUCTION

Broilers are like other domestic birds believed to be susceptible particularly to oxidative stress, likely due to

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the genetic selection towards large breast muscles, increased total weight, and faster growth rate (Sihvo et al., 2013). Broiler meat has been traditionally recognized as highly sensitive to oxidative process due to high degree of unsaturated muscle lipids (Min et al., 2008). Excess free radicals resulting from natural metabolism can damage important biological activities.

Lipid oxidation in meat and fish-products leads to rancidity, off-flavor and many harmful substances (Sen and Mandal, 2017). Oxidation increases with high intake of oxidizable lipids peroxidation, deterioration of sensitive poly unsaturated fatty acids and low intake of antioxidant nutrients (Morrissey et al., 1998; Smet et al., 2008). Oxidative reaction happening in muscle after postmortem causes deterioration of meat quality during storage. High levels of unsaturated fatty acids in poultry meat have high levels of poly unsaturated fatty Acids (PUFA) which makes the poultry meat more susceptible to oxidation process than beef or pork; so, poultry diet has to be supplemented with antioxidant for optimal growth performance, reproduction and meat quality (Delles et al., 2014). The need in natural antioxidant in poultry industry in recent years has been on the increase due to synthetic antioxidants (butylated hydroxyanisole, butylated hydroxytoluene), and their use in stimulating the occurrence of various chronic diseases in humans, animals and birds which prohibited their use.

The alternative to synthetic antioxidants are natural ones which are safer, cheaper, and can prevent oxidative reactions in products during storage; they do not cause metabolic diseases in animals and birds (Caleja et al., 2017). One of the approaches to increase the oxidative stability of meat is to add antioxidant during the period of feeding or directly during processing (Rojas and Brewer, 2007). Polyphenol compounds, especially, flavonoids, have received an important attention because of their antioxidant activities *in vitro* systems. However, it has been shown that flavonoid compounds are poorly absorbed in the gut and their concentrations in target tissues are too low to perform an effective antioxidant defenses (Surai, 2013). Additionally, nanoparticles are studied as nutrition supplements of diets for improvement of broilers health and performance since they are able to carry nutrients directly to the cell (Elkloub et al., 2015). Recorded nanoparticles, such as silver, increased the weight of small intestine and liver but had no effect on heart, gizzard, and proventriculus of broilers (Felehgari et al., 2013). Silver nanoparticles reported to have a negative effect on liver weight relative to live body weight of broilers (Andi et al., 2011).

The antioxidant activities of components of onion and chitosan have been reported (Lampe, 1999; Swiatkiewicz et al., 2015). Onion and garlic possess well defined antioxidant activity (Stajner and Varga, 2003) corollary with the presence of efficient antioxidant enzymatic system such as superoxide dismutase, catalase and glutathione- S- transferase as well as antioxidant from radical scavengers in erythrocytes (Rai et al., 2009). Garlic powder showed no effect on the relative liver, gizzard and heart (Issa and Abo, 2012) with unaffected spleen when broilers were treated with herbal treatments of garlic, thymus and coneflower (Rahimi et al., 2011).White Mini broilers relative weight of various organs, such as liver, spleen, bursa of fabricius etc. remained unaffected by the onion dietary treatments (An et al., 2015), with a report (Aditya et al., 2017) indicating the relative weight of liver, heart, spleen etc. were not affected by the dietary onion extract. Jakubcova et al. (2014) reported owning to higher concentration of garlic extract in feed ratio, the antioxidant status of chicken has increased; in addition, garlic effective antioxidant activity *in vivo* and *in vitro* has been reported (Jackson et al., 2002). The weight of visceral organs did not show changes when broilers were fed on low percentage of dietary chitosan (Khambualai et al., 2008). Chitosan was reported to improve plasma antioxidant enzymes in broiler chickens (Osho and Adeola, 2020). The current study investigated the effect of garlic and onion extract chitosan nanoparticles on antioxidant enzymes activities and relative weight of visceral organs of rainbow rooster indigenous chicken**.**

MATERIALS AND METHODS

Study location

The rearing of the experimental chicken, visceral organs weight measurement and sampling of meat (thigh and breast muscles) after sacrificing the chicken took place in Jomo Kenyatta University of Agriculture and Technology (JKUAT) at Safari Animal Research Facility; whilst the nanoparticles preparation, and the antioxidant enzymes analysis were done at Pan African University of Science Technology and Innovations (PAUSTI)- Molecular Biology and Biotechnology Laboratory in (JKUAT)-Kenya.

Preparation of aqueous onion and garlic, total phenol and ajoene rich extracts

Onion (*Allium cepa* L.) and garlic (*Allium sativum*) were bought from a market in Juja of Kiambu County. Onion (red *Creole orallium*) and garlic (softneck) were identified by the Botany Department at Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya. The aqueous extract of garlic preparation was done according to the method described by Huzaifa et al. (2014) with modification. 50 g of peeled bulbs of garlic was chopped, ground, mixed with 500 ml distilled water, stored for 24 h. It was then filtered the following day using filter paper (Whatman No. 1) and finally stored at 4°C for use. The ajoene rich extract was prepared according to Viswanathan et al. (2014) with modification. 50 g of peeled garlic bulbs was chopped and homogenously blended with 500 ml of cold distilled water, well stirred, filtered with cotton cloth. Then it was transferred in a new flask containing ethyl acetate, stirred and allowed to stabilize for separation of Ajoene rich extract. The upper layer was pipetted into a filter paper (Whatman No. 1) to remove all the water and collection of semi aqueous substance which is the ajoene rich extract. Hence, ethyl acetate was evaporated and the extracted product was stored at 4°C for use. Preparation of aqueous extract of onion was done according to (Oyebode and Fajilade, 2014) with some modification. The onion bulbs together with the outer part were cleaned with ethanol to remove dirt, and chopped into smaller and thin slices; then they were air dried for 2 weeks and 50 g was weighed. 500 ml of distilled water was added and heated at 72°C for 3 h and allowed to cool at room temperature. The extract was then filtered with filter paper (Whatman No. 1), and then stored at 4°C ready for use. Preparation of total phenol extract was done according to Mujic et al. (2009) with some modification. 50 g of chopped, air dried onion bulb was soaked in 500 ml of 70% ethanol. It was left over night and filtered the following day by filter paper (Whatman No. 1); and the ethanol solvent was completely removed by rotary evaporator under vacuum at a temperature of 55°C and the extract was stored at 4°C for use.

Determination of total phenolic and flavonoids content from garlic (softneck) and onion (red *Creole orallium*)

The amount of total phenolic content was determined by the Folin-Ciocalteu method as described by Ainsworth and Gillespie (2007) with modification. 5gm of dried samples of garlic and onion were weighed into separate 250 ml conical flask and about 100 ml ethanol was added. The flask was closed safely using parafilm and covered with aluminum foil. Then, the samples were placed in a shaker and shaken for about 3 h and the extract was kept in darkness for 72 h for further extraction and filtration using filter paper (Whatman No. 4). The extract was topped to 50ml using ethanol; hence, centrifuged for 10 min at 25 degrees at 150 rpm. 1ml was taken from the supernatant and filtered using 0.45 µm micro filter for total phenolic content determination. Then it was placed in a test tube of 2 ml folin ciocateu 10%; 4 ml of 0.7M sodium carbonate was added and vortexed again, then left for 2 h to develop color. It was read in the Uv-vis at 765nm using gallic acid as a standard. Aluminum chloride colorimetric method was used for determination of flavonoids (Jagadish et al., 2009). In 10 ml volumetric flask 4 mL of distilled water and 1 ml of the filtered plant extract were added. After 3 min, 0.3 mL of 5 % sodium nitrite solution was added and after 3 min again, 0.3 ml of 10% aluminum chloride was added. After 5 min, 2 ml of 1 M sodium hydroxide was added and the volume made up to 10 mL with water. Absorbance was measured at 415 nm using UV-Vis spectrophotometer (Shimadzu model UV – 1601 PC, Kyoto, Japan). The amount of total flavonoids was calculated from calibration curve of standard prepared from quercetin (Figures 1 and 2 and Table 3).

The preparation of chitosan solution, chitosan nanoparticles and characterization

Chitosan solution was prepared as described by Rasaee et al. (2016) with some modifications. The low molecular weight chitosan was purchased from Sigma Anderia; 2 g (w/v) was used with 0.5% (v/v) acetic acid and the pH of the solution was raised to 5 with 1N NaOH under magnetic stirring for 24 h and topped to a volume of 200ml with distilled water, and stored at 4°C till application. Preparation of chitosan nanoparticles was done through ionic gelation interaction between positive and negative charged compounds as described by Rasaee et al. (2016) with some modifications. Aqueous chitosan was prepared by mixing 40 ml of chitosan solution with 10 ml of garlic and onion aqueous extract (CHIAGO) mixture as treatment 1, and a mixture of 10 ml of total phenol and homogenous ajoene rich extract with 40 ml chitosan solution (CHITPA) as treatment 2. The mixtures were stirred for 10 min at 60°C, centrifuged at 200 rpm and allowed to rest at room temperature for 30 min to form an opalescent solution. The nanoparticles prepared from aqueous garlic and onion, total phenol and ajoene rich extracts were characterized by their sensitivity to pH to confirm the formation of the nanoparticles, and Fourier

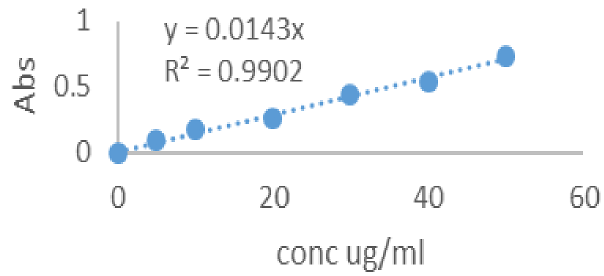

Figure 1. Standard curve for total phenolic content.

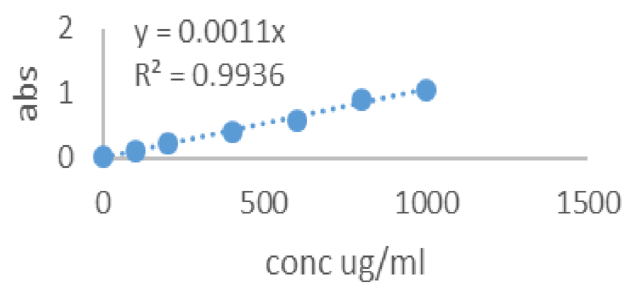

Figure 2. Standard curve for total flavonoid content.

Transform Infrared Spectroscopy (FTIR) to identify the functional groups responsible for the formation of the chitosan nanoparticles of chitosan with the extracts of garlic and onion. Field Emission Scanning Electron Microscopy (SEM) analysis was done to confirm the morphology and size of the nanoparticles prepared (Tables 4 and 5).

Chicken husbandry, treatment and samples collection

Seventy-two chickens were selected from a mixed population of indigenous Rainbow Rooster Chickens aged 8 weeks which were procured from Kukuchic Company Ltd in Nairobi. The chickens were reared on wooden cages in Jomo Kenyatta University of Agriculture and Technology (JKUAT) at Safari Animal Research Facility for a period of 8 weeks. Nine chickens were placed in one treatment with three chickens per cage and three replicates. The chickens were treated with four treatments at 5% and 10% of CHISOLN, CHIAGO, CHITPA and 0.5 g and 1 g of Fosbac orally administered 2 times a week for a period of 8 weeks with the control group placed on water and feed. Food was provided *ad libitum* for both control and treatment groups; the chickens were fed on Kienyeji grower mash produced from a commercial feed company (First Animal Feed) (Tables 1 and 2). The chickens were acclimatized for two weeks followed by oral administration of the treatments for a period of 8 weeks. One chicken from each group and control were sacrificed by method of cervical dislocation according to Laudadio et al. (2012). One chicken from the four treatments with different concentrations and a control was sampled for determination of relative weight of visceral and antioxidant enzymes activities. 72 chickens (9 each week) were analyzed from 1st to 8th weeks relative weight of visceral organs and CAT activity of meat (thigh and breast muscles) stored at -20°C and 27 samples (9 each week) was analyzed for DPPH inhibition of meat (thigh and breast muscles) for 1st week, 4th week and 8th week of the treatment.

Table 1. Basic diet composition.

Feed ingredient	Quantity(kg)
Maize	50
Maize bran	400
Wheat bran	250
Wheat pollard	171
Fish meal	30
Sunflower seed cake	30
Cotton seed cake	20
Stock lime	30
Bone meal	11
Calcium phosphate	2
Methionine	0.5
Coccidiostat	0.5
Grower pre-mix	2
Red salt	3
Total	1000

Table 2. Chemical composition.

Dry matter (%)	92
Crude fiber (%)	11
Metabolisable energy (kcal/kg)	2435.904
Crude protein (%)	14
Calcium (%)	3.75
Total phosphorus (%)	0.34

Table 3. Total phenolic and flavonoid content in softneck garlic and red *Creole orallium* onion.

Item	Solvent used for extraction	TPC (mg QAE/100 g of extract)	TFC (mg QCE/ 100 g of extract)
Onion	70% Ethanol	46.791±1.486	151.828 ± 7.195
Garlic	70% Ethanol	81.606±2.698	260.913 ± 17.113

GAE gallic acid equivalent; QCE quercetin equivalent; TFC total flavonoid content; TPC total phenol content.

Table 4. Composition of chitosan nanoparticles encapsulated with total phenols and ajoene rich extract (CHITPA).

Chitosan nanoparticles prepared	Total phenolic and flavonoid content / 5 ml of onion extract	Ajoene rich extract in/ 5 ml	40 ml	50 ml
(CHITPA)	0.2339 mg TPC and 0.7591 mg TFC	0.5 mg	Chitosan solution	Chitosan nanoparticles

Table 5. Composition of chitosan nanoparticles encapsulated with aqueous of garlic and onion (CHIAGO).

Chitosan nanoparticles prepared	Aqueous of garlic	Aqueous of onion	40 ml	50 ml
(CHIAGO)	5 ml	5 ml	Chitosan solution	Chitosan nanoparticles

Feed composition

Determination of catalase antioxidant activities in meat muscle and blood

The catalase assay was determined by Jenway model 68 spectrophotometer at 240 nm by measuring the disappearance of H_2O_2 with bubble formation; it was characterized by a decrease in absorbance at 240 nm three times for each 30 s according to a modified version of a method described by Aebi (1984) and Babiker et al. (2016). 1 cm quartz cuvette was used with 5g sample of meat muscle mixed with 25 ml of 50 mM phosphate buffer (pH 7.0 at 25°C) using a homogenizer for 15s at 13,500 rpm. The mixture was then centrifuged at 1,800×*g* and 2°C for 15 min. The supernatant of the mixture was taken and filtered through a filter paper (Whatman' No. 1); then, 1 ml of filtered supernatant was mixed with 2 ml of 10 mM H_2O_2. The decrease in absorbance at 240 nm was recorded every 30 s for 1 min. The CAT activity was expressed for meat muscle, liver and erythrocytes as unit/g and unit/ ml of samples, respectively. Measuring CAT absorbance in blood erythrocytes; blood samples of 4 ml of blood was collected from the brachial vein of each chicken and placed into 5 ml aseptic EDTA tubes. The blood samples were centrifuged immediately at 1,370 rpm and 4°C for 10 min; the plasma was separated and erythrocyte was lysed with distilled water (1: 1 v/v). It was inverted vigorously, and centrifuged at 4,020 rmp and 4°C for 15 min. The erythrocytes lysate was collected for the measurement of CAT by adding 1ml of 10 mM H_2O_2, 1ml PBS and 0.1 ml of the sample. In measuring CAT in liver, the liver organ was washed thoroughly in ice-cold physiological saline and weighed. Homogenate of 10% was prepared as described by Patlolla et al. (2009) with modifications; 2.5g liver was put in 25 ml of 0.05 M phosphate buffer (pH 7.4) containing 0.1 mM EDTA; the sample was homogenized, followed by sonication and centrifugation at 4,000 rpm and 4°C for 10 min. The supernatant was decanted and centrifuged at 16000 rpm for 30 min at 4°C. The supernatant fraction was obtained and called "homogenate; it was used to measure CAT by adding 2ml of 10 mM H_2O_2, and 0.1 ml of the sample. The extension coefficient of 40 M-1 cm-1 for H_2O_2 at 240 nm was used to calculate catalase enzyme activities as per Babiker et al. (2016).

$$\text{Catalase activity (umole/ ml)} = \frac{\text{Decrease in absorbance of hydrogen peroxide at 240 nm}}{\text{Molar extension coefficient of hydrogen peroxide at 240 nm}}$$

One unit of catalase activity is defined as the amount of activity required to convert 1µmole/ ml (umole/ ml) of hydrogen perioxide to water and oxygen per minute at 25°C.

Determination of free radical scavenging activity of thigh and breast muscle

Radical scavenging activities of the chicken's thigh and breast muscle sample stored at -20°C with 2, 2-Diphenyl-1-picryl hydroxyl (DPPH) free radical (Sigma-Aldrich) were determined by UV spectrophotometer at 517 nm according to the method of Molyneux (2004). One gram of fresh meat stored at -20°C was weighted and socked into a 100 ml Falcon tubes; 25 ml methanol was added.

The sample in Falcon tube was covered with aluminum foil. The sample was placed in a shaker and stirred for about 3 h and then kept in darkness to extract for 72 h. The sample was filtered through filter paper (Whatman No. 4), and 1:1 serial dilution was made by picking 1 ml out of 25 and 1 ml of methanol as first concentration (40 mg/ml); the dilution continued 5times and the following concentrations of the extracts were prepared, 40, 20, 10, 5 and2.5 mg/ml in methanol (Analytical grade). Vitamin C was used as the antioxidant standard at the same concentration as the extract, that is 1 g into 25 ml of methanol and 5 dilution of 1:1. One ml (1 ml) of the extract was placed in a test tube, and 3 ml of methanol was added followed by 0.5 mL of 1 mM DPPH in methanol. A blank solution was prepared containing the same amount of methanol and DPPH. Methanol was used to zero the spectrophotometer and the absorbance was read at 517 nm after 5 min in UV-Vis spectrophotometer (Shimadzu model UV – 1601 PC, Kyoto, Japan); the radical scavenging activity was calculated using the following formula:

$$\% \text{ inhibition of DPPH} = \{(A_B - A_A)/A_B\} \times 100$$

Where A_B is the absorption of blank sample and A_A is the absorption of tested extract solution. The results were expressed as percentage inhibition of DPPH.

Statistical analysis

Graphpad Statistical Package version 7.1 was used to draw graphs and the data analyzed statistically using Tukey mean differences in Statistical Analysis System (SAS) statistical software version 9.1; statistical significant difference was considered at $p<0.05$.

RESULTS

Determination of active components from garlic and onion and the composition of garlic and onion chitosan nanoparticles prepared

Effect of Garlic and onion chitosan nanoparticles on body weight and relative weight of visceral organs

Compared to the control, all treatments had increase significantly (p=0.0001) with a decrease observed in CHIAGO 10% treatment in the chicken body weight (Figure 3). Treatment with 0.5 g Fosbac, 5% CHIAGO and CHSOLN 10% significantly increased the relative weight of the gizzard (Figure 6) in comparison to the control ($p<0.05$). The relative weight of the heart (Figure 4), liver (Figure 5) and the spleen (Figure 7) were not significantly different from the control group ($p>0.05$).

Catalase enzyme activity in erythrocytes, liver, thigh and breast muscle

The CAT activity in thigh muscle was significantly increased by all the treatments (p=0.0027) (Figure 8). In the breast muscles CAT activity was significantly (p=0.0108) increased by all the treatments (Figure 9). CAT activity in erythrocytes (Figure 10) and liver (Figure 11) have no significant changes ($p>0.05$).

Free radical scavenging activity of thigh and breast muscle of Rainbow rooster chicken

Vitamins C (Vit C) showed high DPPH inhibition%. CHIAGO 10%, CHITPA 10% showed higher inhibition% in week 4 and week 8 compared to the other treatments;

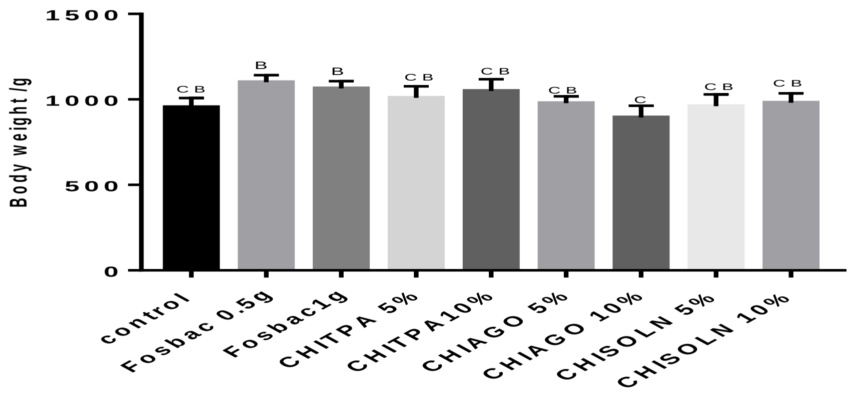

Figure 3. Mean body weight of chicken (P value = 0.0001).

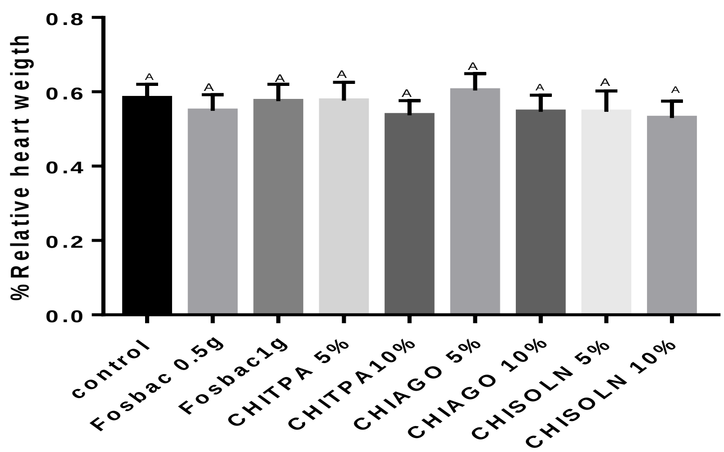

Figure 4. Mean % relative heart weight of chicken (P value = 0.1812).

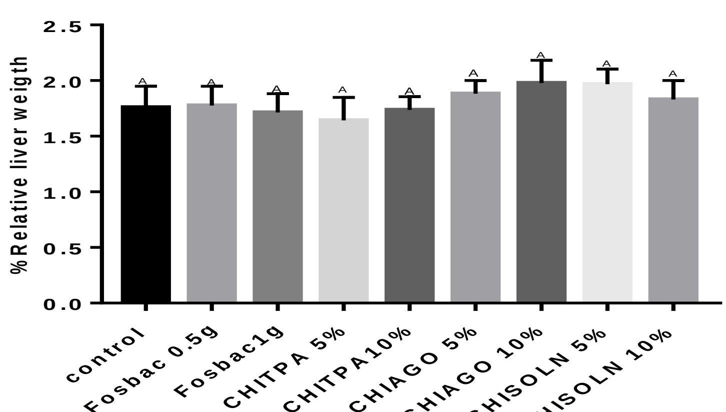

Figure 5. Mean % relative liver weight of chicken (P value = 0.7315).

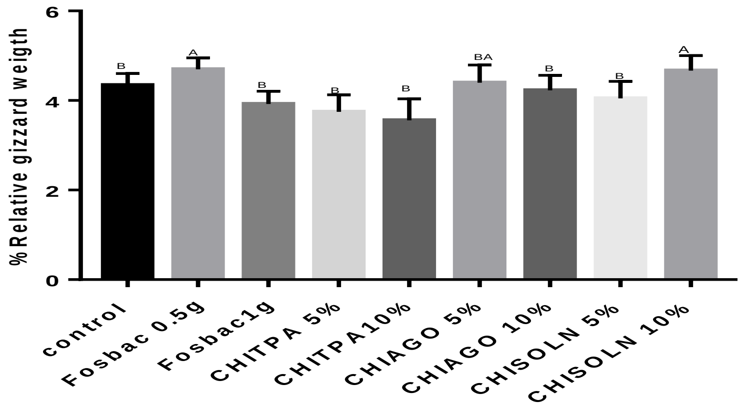

Figure 6. Mean % relative gizzard weight of chicken (P value = 0.0002).

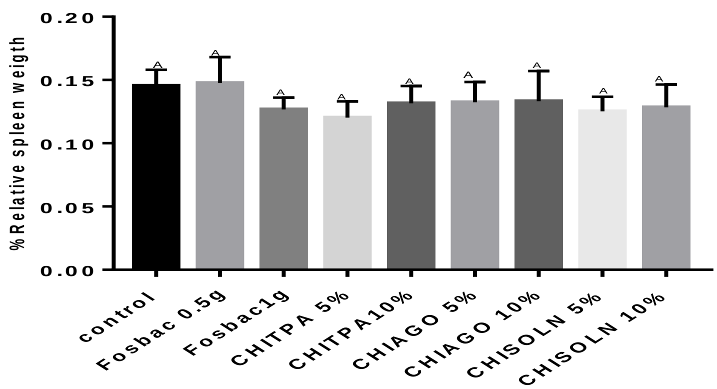

Figure 7. Mean % relative spleen weight of chicken (P value = 0.3545).
Control group is with only water and feed; Fosbac is antibiotics (control positive); CHISOLN is Chitosan solution; CHIAGO is chitosan with aqueous of garlic and onion; CHITPA is Chitosan with total phenol and ajoene rich extract. Means with the different letters are significant different at (*P* 0.05). Standard error of the mean (SEM) (n=8).

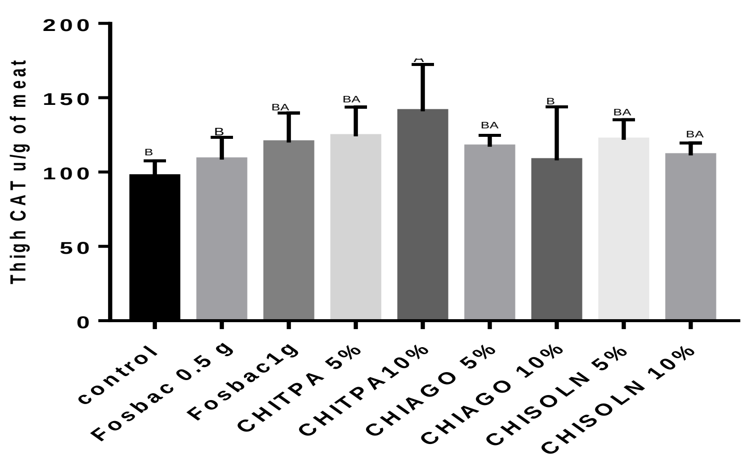

Figure 8. Mean CAT activities of chicken thigh (P value = 0.0027).

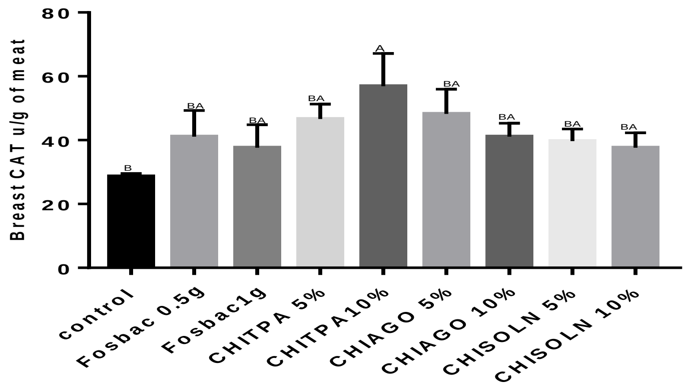

Figure 9. Mean CAT activities of chicken breast (P value = 0.0108).

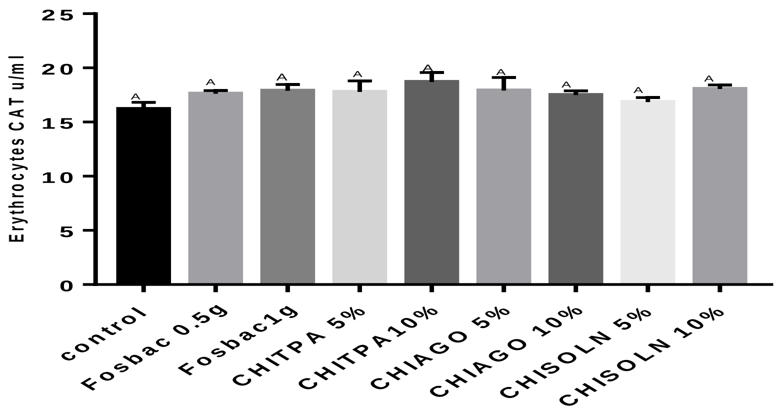

Figure 10. Mean CAT activities of chicken erythrocytes (P value = 0.3526). Control group is with only water and feed; Fosbac is antibiotics (control positive); CHISOLN is Chitosan solution; CHIAGO is chitosan with aqueous of garlic and onion; CHITPA is Chitosan with total phenol and ajoene rich extract. Means with the different letters are significant different at (P 0.05). Standard error of the mean (SEM) (n=8).

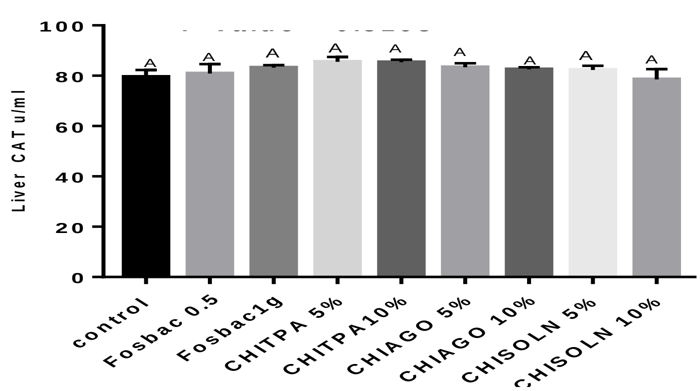

Figure 11. Mean CAT activities of chicken liver (P value = 0.3198) Control group is with only water and feed; Fosbac is antibiotics (control positive); CHISOLN is Chitosan solution; CHIAGO is chitosan with aqueous of garlic and onion; CHITPA is Chitosan with total phenol and ajoene rich extract. Means with the different letters are significant different at (P 0.05). Standard error of the mean (SEM) (n=8).

in week 1, CHIAGO 5%, CHIAGO 10% had higher scavenging activities in thigh, in breast, the treatments, 1 g of Fosbac and CHITPA 5% had a higher activity when compared with other treatment and the control chicken (Figures 12 to 17).

DISCUSSION

Garlic and onion extract chitosan nanoparticles effect on relative weight of visceral organs

The relative weight of heart, liver and spleen from chicken in the treatment group did not change significantly when compared with those in the control group ($p>0.05$). The result is comparible with reports of Issa and Abo (2012), An et al. (2015) and Khambualai et al. (2008) and also with agreement with the findings of of Pourmahmoud et al. (2013) who reported no effect on the internal organs when thyme extract was supplemented in broilers. Similarly, Abo et al. (2016) revealed that herbal supplementation had no significant effects on some visceral organs. There is a significant increase ($p<0.05$) in gizzard for a few treatments correlate with the findings of Aguzey and Gao (2018) who reported that feeding broilers on mash diets have positive effect on gizzard development than feeding pelleted diets by increasing the relative weight. The result also corrlates with research finding on Hawthorn plant extract having content of flavoniods; when added to the drinking water of broilers it reduces the proportion of the body attributed to abdominal fat, liver and heart (Ahmadipour et al., 2017) and reduces relative liver weight and abdominal fat (Ahmadipour et al., 2018).

Catalase enzyme activity in erythrocytes, liver, thigh and breast meat muscle

Catalase enzyme activities in erythrocytes and liver did not differ significantly ($p>0.05$) when compared with the control chicken. CAT activity though statistically indicated no significant differences in the levels of all treatment were slightly higher than the control in erythrocytes and liver except CHISOLN10% that had the same amount as the control. It correlates with the findings of Kelussia odoratissima medicinal plant extract with flavonoids and polyphenols; when fed to broilers it significantly suppresses hepatic lipogenesis by downregulating key hepatic lipogenic enzyme genes and boosts antioxidant capacity by up-regulating hepatic antioxidative genes SOD1 , catalase in the liver (Ahmadipour et al., 2018). It is likely an indication of catalase as a very important enzyme for protection of cells from the toxic effects of H_2O_2 and radical oxygen species such as superoxide, hydroxyl radical reactive oxygen species generated during metabolism attacks cell components such as DNA, protein and lipid membrane. Sometimes lethal damages may occur in the cells, and those potentially injured are neutralized by antioxidant enzymes such as catalases,

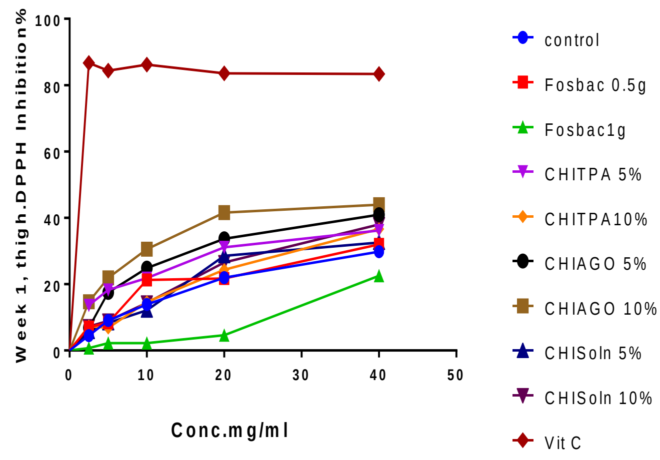

Figure 12. Week1; Conc. DPPH inhibition % of Thigh.

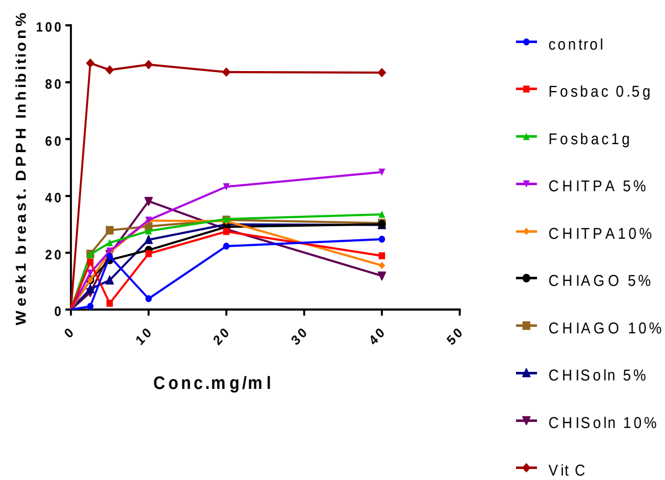

Figure 13. Week1; Conc. DPPH inhibition % of breast.

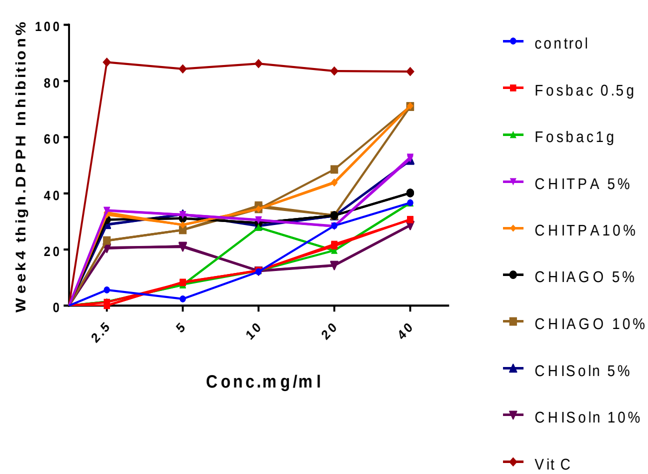

Figure 14. Week4; Conc. DPPH inhibition % of thigh.

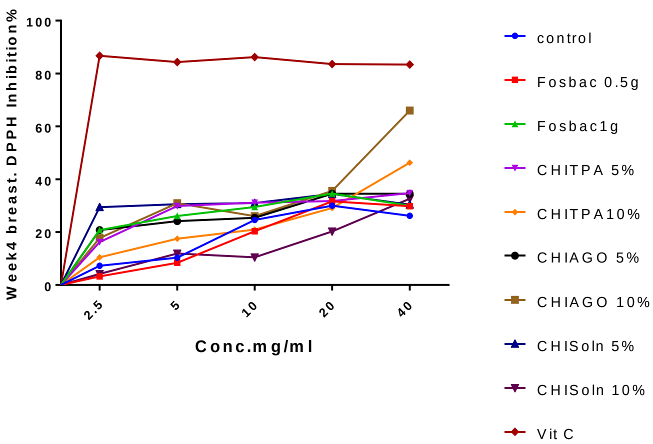

Figure 15. Week4; Conc. DPPH inhibition % of breast.

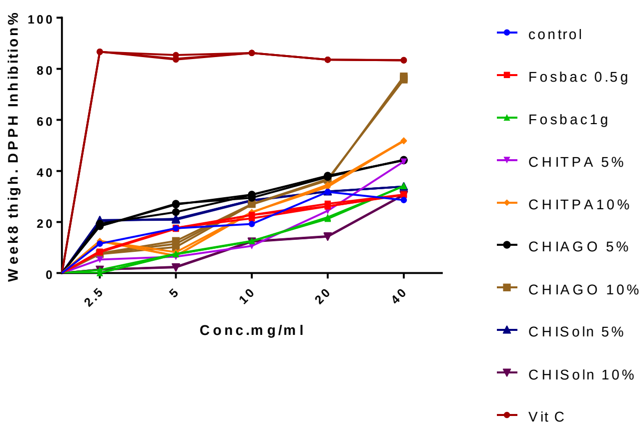

Figure 16. Week8; Conc. DPPH inhibition % of thigh.
Control group is with only water and feed; Fosbac is antibiotics (control positive); CHISOLN is Chitosan solution; CHIAGO is chitosan with aqueous of garlic and onion; CHITPA is Chitosan with total phenol and ajoene rich extract, Vit C (Vitamins C).

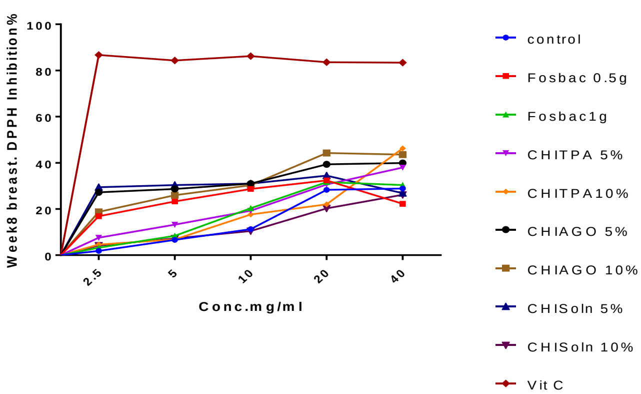

Figure 17. Week 8 Conc. DPPH inhibition % of breast. Control group is with only water and feed; Fosbac is antibiotics (control positive); CHISOLN is Chitosan solution; CHIAGO is chitosan with aqueous of garlic and onion; CHITPA is Chitosan with total phenol and ajoene rich extract, Vit C (Vitamins C).

superoxide dismutase and peroxidases (Aydemir and Kuru, 2003). Chitosan used as feed additive for poultry has an effect on antioxidant properties as reported by Swiatkiewicz et al. (2015). Plant extracts with polyphenols can reduce serum levels of triglycerides and cholesterol as well as abdomnal fat deposition (Ahmadipour et al., 2015). The muscle fibres are categorized into two different metabolic types, oxidative (red) or glycolytic (white), based on the chemical composition and enzyme activities (Warris, 2000).

In this study, thigh and breast muscle antioxidant enzymes activities showed a difference when compared with the control chicken (p<0.05). The result is in agreement with Saleh et al. (2018) who indicated that broiler thigh meat may be enriched successfully with long chain polyunstaturate fatty acids n-3 and its antioxidant potential and functional quality characteristics may be improved by dietary supplymentation.

Renerre et al. (1996) and Muhlisin et al. (2016) indicated that oxidative muscle exhibits higher antioxidant enzyme activities than glycolytic muscles and Lee et al. (1996) reported a higher antioxidant enzyme activities in thigh meat of turkey than breast meat. The activities of antioxidant differ in meat of different animal species (Pradhan et al., 2000; Hernandez et al., 2004). Dellees et al. (2014) reported that the dietary antioxidants can minimize the oxidative instability of proteins and lipids, and the protection may be linked to improved cellular antioxidant enyzymes activities. Catalase and Glutathione peroxidase (GSH-Px) are considered the major peroxide-removing enzymes located in cytosol, whilst superoxide dismutase (SOD) plays a role in the protection against damage resulting from superoxide anion radicals (Chen et al., 2012). SOD and catalase are coupled enzymes in which SOD scavenges superoxide anions by forming hydrogen peroxide, and catalase safely decomposes hydrogen peroxide to water and oxygen; GSH-Px can decompose both hydrogen peroxide and lipoperoxides formed during lipid oxidation (Gatellier et al., 2004; Terevinto et al., 2010).

Free radical scavenging activity in thigh and breast meat

DPPH assay is a rapid, simple and inexpensive, and stable free radical which is widely used to determine antioxidant activities of different biological system. In this study, thigh and breast meat muscles scavenging activities to DPPH was found to be higher in thigh than breast meat of rainbow rooster chicken. There is an increase in antioxidant activities both for thigh and breast at (p<0.05) of the treated groups in comparison to the control groups of chicken. Thigh meat expresses high DPPH inhibition percentage than the breast meat and it showed the inhibition percentage increases as the concentration of the samples increases and the duration of the treatments increases. In the 1st week, thigh with the treatments; CHIAGO 5%, CHIAGO 10% had higher antioxidant scavenging activities. In breast, the treatments; 1 g of Fosbac and CHITPA 5% had a higher activity. In week 4 and week 8; CHIAGO 10%, CHITPA 10% gave high DPPH inhibition percentage both in breast and thigh meat. The result was in comparison with Saleh et al. (2018), who indicated broiler thigh meat may be enriched successfully with long chain polyunstaturated fatty acids n-3 and its antioxidant potential and fuctional quality characteristics may be improved by supplementing the diet. Polyphenols are natural antioxidant that showed antioxidant and animicrobial activities (Lorenzo et al., 2014) and it can prevent lipid oxidation by preventing chain inhibition by scavening initiating radicals, breaking chain reaction, decomposing peroxides, decreasing localized oxygen concentration and binding chain intiating catalyst such as metal ions (Juntachote et al., 2006).

Conclusion

Chitosan nanoparticles of garlic and onion extract do not significantly affect the relative weight of the heart, liver, spleen and gizzard of rainbow rooster chicken. Catalase antioxidant enzyme activities increases with CHIAGO and CHITPA treatments on thigh and breast meat. Thigh and breast meat free radical scavenging increases with increase in the sample concentration and the duration of application of the treatments with CHIAGO 10% and CHITPA 10% showed an increase in the meat muscle antioxidant enzyme activities. Chitosan nanoparticles of garlic and onion extract prepared can be used as a natural antioxidant supplement in rainbow rooster chicken. Further research on the application of the prepared products on the type of breeds and duration of application is advisable.

CONFLICT OF INTERESTS

The authors have declared no any conflict of interest.

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