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Volume 12 (3); May 30, 2022

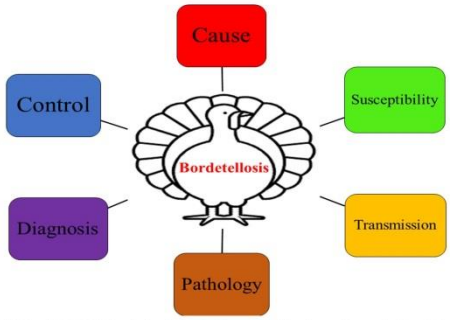
Review

Avian bordetellosis: a significant bacterial respiratory disease of turkeys (*Meleagris gallopavo*)

Abd El-Ghany WA.

Online J. Anim. Feed Res., 12(3): 103-110, 2022; pii: S222877012200014-12
DOI: https://dx.doi.org/10.51227/ojafr.2022.14

Abstract
This review was designed to spotlight on avian bordetellosis regarding the bacterium pathogenesis, susceptibility, transmission, pathology, laboratory diagnosis, and prevention and control measures. Bordetellosis (moreover called turkey coryza) is a contagious bacterial upper respiratory disease of poultry, especially turkey poults. The disease is characterized by high morbidity and low mortality with terrible economic losses for turkeys industry. Bordetellosis is caused by *Bordetella avium* (B. *avium*) bacterium which colonizes and destructs the cilia of the respiratory tract. Concurrent infection during bordetellosis outbreaks is common and contributes to the poor performance of B. *avium*-infected flocks. Domesticated and wild birds are susceptible to bordetellosis. All ages can get infection with bordetellosis, however, young ages are more susceptible than adult. Infection and transmission of B. avium occurs through aerosol, water, and reservoirs, but not vertically. The clinical picture of bordetellosis is usually upper respiratory, except in complications with other bacteria or stressors, the infection extends to the lower respiratory tract. The bacterium is isolated aerobically on 10% sheep blood agar and appears as Gram negative bacilli. Endotoxin, tracheal cytotoxin, heat-labile dermonecrotic toxin, and osteotoxin are produced by B. *avium*. Antibiotic treatment of B. *avium* shows variable results and it is usually ineffective. Different types of living and inactivated vaccines are used to prevent bordetellosis.



Abd El-Ghany WA (2022). **Avian bordetellosis: a significant bacterial respiratory disease of turkeys (*Meleagris gallopavo*).** *Online J. Anim. Feed Res.*, 12(3): 103-110. DOI: https://dx.doi.org/10.51227/ojafr.2022.14

Keywords: *Bordetella avium*, Diagnosis, Domesticated birds, Pathogenesis, Turkey coryza.

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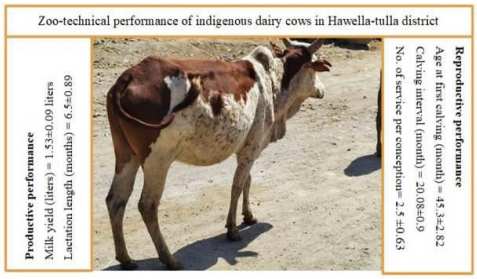
Research Paper

Zoo-technical performance of indigenous dairy cows under small holder farmers management system in Hawella-Tulla district, Ethiopia

Endale S.

Online J. Anim. Feed Res., 12(3): 111-116, 2022; pii: S222877012200015-12
DOI: https://dx.doi.org/10.51227/ojafr.2022.15

Abstract
Ethiopia's dairy production system is predominantly extensive, with poor cattle performance constrained by a variety of factors such as poor genetics, low reproductive performance, and poor quality of feed, among others. So far, little work aimed at increasing dairy production has been undertaken. The objective of the study was to characterize the zoo-technical performance of indigenous dairy cows under small holder farmer management system. The study was undertaken in Hawella-Tulla district, Sidama Region; Sothern Ethiopia from November 2020 to March 2021. From three peasant associations (PA), 180 smallholder dairy farmers who owned local cows were selected using a simple random selection technique. Data were collected using a structured questionnaire. The majority of farmers (75%) practiced in mixed crop-livestock farming primarily produce milk for income generation. Farmers hold an average of 1.95±0.063 local lactating cows. The productive performance of the indigenous cow mean of daily milk yield was 1.53±0.09 liters and 6.5±0.89 months of lactation length. The mean age at first service (AFS) was 38.5 ± 2.71 months, Age at first calving (AFC) was 45.3±2.82 months, calving interval (CI) was 20.08±0.9 months, and number of services per conception (NSPC) was 2.5 ±0.63. It can be concluded that the zoo-technical performance of local cows was very low. Therefore, planned technical and institutional intervention to improve the feeding system, provide better health management, genetic improvement of



Endale S (2022). **Zoo-technical performance of indigenous dairy cows under small holder farmers management system in Hawella-Tulla district, Ethiopia.** *Online J. Anim. Feed Res.*, 12(3): 111-116. DOI: https://dx.doi.org/10.51227/ojafr.2022.15

local breeds through crossbreeding and synchronization should be carried out for the betterment performance of local cows.
Keywords: Breeding, Calving, Indigenous cows, Smallholder system, Zoo-technical performance.

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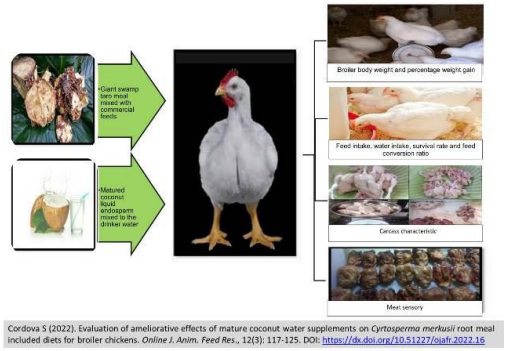
Research Paper

Evaluation of ameliorative effects of mature coconut water supplements on *Cyrtosperma merkusii* root meal included diets for broiler chickens

Cordova S.

Online J. Anim. Feed Res., 12(3): 117-125, 2022; pii: S222877012200016-12
DOI: https://dx.doi.org/10.51227/ojafr.2022.16

Abstract
The simultaneous degradation of essential nutrients is the major drawback in detoxifying anti-nutrient toxic substances of root meal-based diets. An ameliorative dietary supplement for root meal-based diets without undergoing thorough detoxification is proposed. Therefore, this trial aims to determine the effects of mature coconut water (MCW) against the anti-nutritional factors (ANF)-containing *C. merkusii* root meal diet on growth performance, carcass characteristics, and organoleptic traits of broiler chickens. Cobb 500 (*n*=160) day-old (male) grouped into the standard diet (commercial maize-soybean) or ANF (15% raw *C. merkusii* + 85% commercial maize-soybean) diet group, and the birds every group further allocated into 0, 5, 10, and 15% MCW water treatment groups (*n*=5) with four replications. The trial lasted for 20 D (8 to 28 D of age). In the ANF diet, treatment with MCW significantly differs on feed conversion ratio (FCR). The 5-15% MCW treated chickens were more feed-efficient than the 0% MCW treatment. MCW treatments were not significant on body weight, weight gain, survival, carcass component, and organoleptic traits of broiler chickens under the standard or the ANF diets. However, significant diet*water interactions were observed on BW and dress weight, and significant gizzard weight due to diets. In general, the improved FCR may be the ameliorative effect of mature coconut water against ANF on raw *C. merkusii* root meal inclusion (15% + 85%) in the standard diets.
Keywords: Ameliorative effect, Anti-nutritional factor, *Cocos nucifera*, *Cyrtosperma merkusii,* Root meal.



Cordova S (2022). Evaluation of ameliorative effects of mature coconut water supplements on *Cyrtosperma merkusii* root meal included diets for broiler chickens. *Online J. Anim. Feed Res.*, 12(3): 117-125. DOI: https://dx.doi.org/10.51227/ojafr.2022.16

[Full text-PDF] [Export citation to RIS & EndNote] [How to Cite]

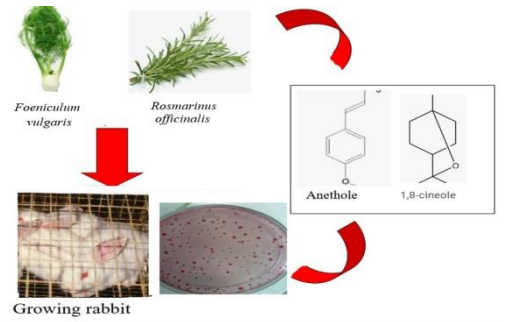
Research Paper

The effect of dietary supplemented dried fennel and rosemary on the performance and caecal microflora of growing rabbits

Benlemlih M, Barchan A, Aarab A, Bakkali M, Arakrak A, and Laglaoui A.

Online J. Anim. Feed Res., 12(3): 126-131, 2022; pii: S222877012200017-12
DOI: https://dx.doi.org/10.51227/ojafr.2022.17

Abstract
The objective of this study is to evaluate the possible effect of fennel and rosemary dietary supplements on the performance of rabbits. Therefore fifty-six weaned rabbits (40 days old) from white New Zealand breed were divided into two groups and submitted to the following dietary treatments: A) control diet and B) FR diet consisted of 2.5% *Foeniculum vulgare* seeds (fennel) and *Rosmarinus officinalis* leaves (Rosmary) as powder mixed by control diet for thirty days. The growth rate, feed conversion ratio, carcass yield, and mortality were not influenced by dietary fennel and rosemary supplementation. The antimicrobial effect of fennel and rosemary (2.5%) was not observed against *E. coli* in the caecum of the rabbit treated. The essential oils of *Foeniculum vulgare* and *Rosmarinus officinalis* are predominantly composed by Trans Anethole and 1-8 cineole successively. Low antibacterial activity was observed with two essential oils against the strain tested in this study. The addition of the 2.5% combination of fennel and rosemary in rabbit feed did not influence the zootechnical parameters of the rabbits
Keywords: Caecal microflora, Fennel, Growth parameters, Rabbit, Rosemary.



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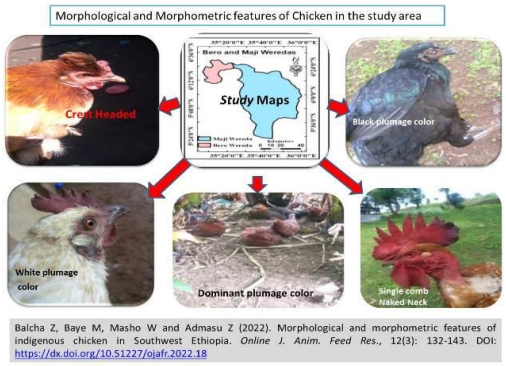
Research Paper

Morphological and morphometric features of indigenous chicken in Southwest Ethiopia

Balcha Z, Baye M, Masho W and Admasu Z.

Online J. Anim. Feed Res., 12(3): 132-146, 2022; pii: S222877012200018-12
DOI: https://dx.doi.org/10.51227/ojafr.2022.18



Balcha Z, Baye M, Masho W and Admasu Z (2022). Morphological and morphometric features of indigenous chicken in Southwest Ethiopia. *Online J. Anim. Feed Res.*, 12(3): 132-143. DOI: https://dx.doi.org/10.51227/ojafr.2022.18

Abstract
Morphological and morphometric characterization of indigenous chicken ecotypes were carried out in West-Omo zone of Southwest Ethiopia. Nine qualitative and fifteen quantitative traits were observed/ measured from 660 matured chickens of both sexes. The data was analysed using SPSS version 21 and SAS version 9.1. Majority of the qualitative and quantitative traits were significantly influenced by sex and agro-ecological zones. The predominant plumage color, feather distribution, shank color, skin color, ear-lobe color, eye color, head shape, comb type, and feather morphology were red (38.4%), normal (96.2%), yellow (45%), white (48.8%), red (42.1%), red (28.6%), flat plain (94.4%), single (64.7%) and normal (100%). The body weight of matured male chickens in highland, mid-altitude and lowland agro-ecologies was 2.1±0.02 kg, 2.2±0.05 kg, and 2.0±0.03 kg, respectively, while females weighed 1.4±0.01 kg, 1.5±0.00 kg, and 1.4±0.01 kg in highland, mid-altitude, and lowland agro-ecologies, respectively. Males were also superior to females in terms of body length (BL) values of 42.0 ± 0.19cm and 37.7 ± 0.06cm, respectively. The prediction of body weight could be based on regression equation y = -1.02 + 0.10 CC (chest circumference) for male and y= -1.26 + 0.07BL of hen in highland altitude, y = -1.06+ 0.11 CC of male and y= -0.78+ 0.05 BL of hen in mid-altitude and similarly y = -0.90 +0.10 CC in lowland male and y= -1.33 + 0.07 BL of lowland hen. Therefore, chest circumference for males and body length for females were the best variables to predict the body weight of chickens than other variables. The current finding shows there was heterogeneity in a population of indigenous chickens in the studied agro-ecology. This gives an opportunity for genetic improvement of indigenous chickens within a population.
Keywords: Genetic improvement; Indigenous chicken; Morphological; Morphometric; West-Omo zone.

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Research Paper

The importance of the feeding levels and adequacy on the meat quality and productivity performance of cross-bred bulls

Velmatov AA, Tishkina TN, Neyaskin NN, Velmatov AP.

Online J. Anim. Feed Res., 12(3): 147-153, 2022; pii: S222877012200019-12
DOI: https://dx.doi.org/10.51227/ojafr.2022.19

Velmatov AA, Tishkina TN, Neyaskin NN, Velmatov AP (2022). **The importance of the feeding levels and adequacy on the meat quality and productivity performance of cross-bred bulls.** *Online J. Anim. Feed Res.*, 12(2): 144-151. DOI: https://dx.doi.org/10.51227/ojafr.2022.19

Abstract
The study aimed to determine the effect of the level and adequacy of feeding on the dynamics of live weight and meat productivity of Simmental × Holstein bulls with a close blood relationship (87.5%) for the Holstein breed. Three groups of bulls with 87.5% Holstein heredity in the genotype were formed, out of which two groups were experimental and one group served as a control group. The bulls of the control group received a diet compiled according to detailed standards, and their analogs from the experimental groups had a diet exceeding the norm by 10 and 20% respectively. During the entire growing period, the bulls of the 1st experimental group consumed 3,564 energetic feed units and 362.5 kg of digestible protein, the 2nd consumed 3,875 and 394.3 kg, and the control group 3,245 and 329.9 kg, respectively. In terms of carcass yield, bulls of the first and second experimental groups outperformed the ones from the control group by 2.41 and 3.92%. With an increase in the level of feeding in experimental bulls, fat deposition accelerated; thus, the meat of bulls of the experimental groups contained 2.13% and 2.54% more fat than in animals of the control group. At the same time, the protein content in the meat of experimental bulls decreased by 0.15 and 0.22% in comparison with the control group. The energy value of 1 kg of meat of experimental bulls was higher by 0.77-0.90 MJ. It can be concluded that a possible increase in the meat productivity of bulls-crossbreeds of the Holstein breed with a close blood relationship and the determination of planned indicators of animal growth should be taken into account when developing breeding programs.
Keywords: Breed, Bulls, Carcass yield, Genotype, Live weight.

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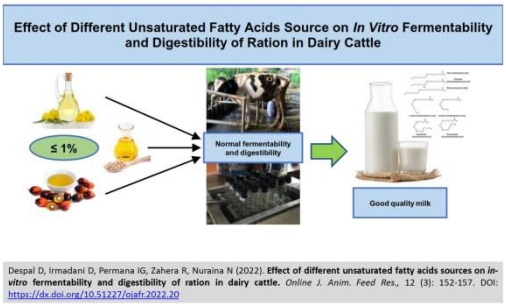
Research Paper

Effect of different unsaturated fatty acids sources on in-vitro fermentability and digestibility of ration in dairy cattle

Despal D, Irmadani D, Permana IG, Zahera R, Nuraina N.

Online J. Anim. Feed Res., 12(3): 154-159, 2022; pii: S222877012200020-12
DOI: https://dx.doi.org/10.51227/ojafr.2022.20



Abstract
Supplementation of oil rich in unsaturated fatty acids (FAs) such as canola, soybean, and palm oils improved the quality of milk fatty acids. However, the unprotected unsaturated oil might impair rumen fermentation, feed, and fiber digestibility. A study was conducted to determine the best type of oil supplementation (factor A) including canola (A1), soybean (A2), or palm (A3) and level oil supplementation (factor B) including B0 = 0%, B1 = 1%, B2 = 2% or B3 = 3%) on the *in-vitro* feed fermentation and digestibility. The study used a 3 x 4 factorial block design. Two-stages were used to measure the pH, ammonia (NH3), volatile fatty acids (VFAs), protozoal number, dry matter (DMD), organic matter (OMD), neutral detergent fiber (NDFD), and acid detergent fiber (ADFD) digestibility. The results showed that oil type did not significantly influence the fermentability (pH, NH3, VFAs, and protozoa) and feed's digestibility (DMD, OMD, NDFD, and ADFD) but oil level influence the fermentability and digestibility significantly. In addition, an increase above 1% in oil levels reduced protein fermentability, protozoal number, DMD, and OMD, but increased VFA. It is concluded that the addition of unprotected canola, soybean, or palm oil in dairy cattle ration could be applied in a concentration not more than 1%.
Keywords: Canola oil, Milk fatty acid, Palm oil, Ration, Soybean oil.

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Research Paper

Molybdenum supplementation of fat-tailed ewes diets in an arid region

Gayirbegov DS, Mandzhiev DB, Tyurbeev TB.

Online J. Anim. Feed Res., 12(3): 160-164, 2022; pii: S222877012200021-12
DOI: https://dx.doi.org/10.51227/ojafr.2022.21

Abstract
The study aimed to establish the normal molybdenum requirements for open ewes of meat-and-fat breeds in arid climatic conditions. The study was carried out in the production conditions of the Buddha farm of the Republic of Kalmykia (Russia). The norm has been established based on a detailed study of the molybdenum content in organs and tissues and the degree of its absorption from the diet, considering the endogenous losses. It has been established that the norm of molybdenum is 4.5 mg per head per day, 2.8 mg per 1 kg of dry matter of the diet and 0.80 mg per 1 kg of live weight of a single ewe. Recommendations have been made to solve the problem of molybdenum deficiency in the diet of open ewes of meat-and-fat breeds in arid climatic conditions of Republic of Kalmykia.
Keywords: Open ewes, Norm, Element, Concentration, Kalmyk breed

[Full text-PDF] [Export citation to RIS & EndNote] [How to Cite]

Research Paper

Traditional Management Practices and Production Potential of Beekeeping in Erer Zone of Somali Regional State, Ethiopia

Mahamed A, Abdimahad K, Abdilahi A, Hassen G, Hassen M, Omer A.

Online J. Anim. Feed Res., 12(3): 165-172, 2022; pii: S222877012200022-12
DOI: https://dx.doi.org/10.51227/ojafr.2022.22

Abstract

The study was carried out in Erer zone, Somali Region of Ethiopia, to assess beekeeping practices and production. A total of 156 respondents were selected randomly from two purposively selected localities based on beekeeping potential and interviewed using semi-structured questionnaire. The study revealed the majority of the respondents (85.9%) practiced traditional beekeeping system and hung their hives on trees away from the homestead in dense forest. The honeybee flora of the area consists mostly of nectar and pollen-producing trees and shrubs including *Grewia tenax*, *Grewia penicillata*, *Acaciamellifera*, *Acaciareficiens*, *Acaciatortilis*, *Acaciasenegal*, and *Acaciahorrida*. The average honey yield from traditional beehives was 4.85 kg, which is less than the national average yield (5 kg). The average honey yield from modern beehives, on the other hand, was 7.29 kg which is lower the amount obtained from other parts of the country. The major constraints of beekeeping are the high cost of modern hives and accessories, pests and predators of honey bees, water scarcity, shortage of bee forage, bee absconding and marketing. Birds, ants, spiders, wax moth, mice, lizards, and honey badgers were identified as the major honeybee pests and predators based on beekeeper responses. Pests and predators (37.8%), destroying nests during honey harvesting (26.9%), water scarcity (21.2%), and shortage of bee forage (14.1%) were the most common reasons for honeybee absconding. Beekeeping production remains low due to these constraints and traditional practices in the area. However, there is enormous potential and opportunity to increase honeybee production in the area. To improve the quantity and quality of honey yield in the area, significant extension and technical intervention, use of locally available beekeeping technologies, appropriate measures to manage honeybee pests and absconding, and training to enrich beekeepers' knowledge are necessary to be implemented.

Keywords: Bee forage, Beekeeper, Beekeeping practice, Hive, Honey production.

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AVIAN BORDETELLOSIS: A SIGNIFICANT BACTERIAL RESPIRATORY DISEASE OF TURKEYS (*Meleagris gallopavo*)

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Supporting Information

REVIEW ARTICLE

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ABSTRACT: This review was designed to spotlight on avian bordetellosis regarding the bacterium pathogenesis, susceptibility, transmission, pathology, laboratory diagnosis, and prevention and control measures. Bordetellosis (moreover called turkey coryza) is a contagious bacterial upper respiratory disease of poultry, especially turkey poults. The disease is characterized by high morbidity and low mortality with terrible economic losses for turkeys industry. Bordetellosis is caused by *Bordetella avium* (*B. avium*) bacterium which colonizes and destructs the cilia of the respiratory tract. Concurrent infection during bordetellosis outbreaks is common and contributes to the poor performance of *B. avium*-infected flocks. Domesticated and wild birds are susceptible to bordetellosis. All ages can get infection with bordetellosis, however, young ages are more susceptible than adult. Infection and transmission of *B. avium* occurs through aerosol, water, and reservoirs, but not vertically. The clinical picture of bordetellosis is usually upper respiratory, except in complications with other bacteria or stressors, the infection extends to the lower respiratory tract. The bacterium is isolated aerobically on 10% sheep blood agar and appears as Gram negative bacilli. Endotoxin, tracheal cytotoxin, heat-labile dermonecrotic toxin, and osteotoxin are produced by *B. avium*. Antibiotic treatment of *B. avium* shows variable results and it is usually ineffective. Different types of living and inactivated vaccines are used to prevent bordetellosis.

Keywords: *Bordetella avium*, Diagnosis, Domesticated birds, Pathogenesis, Turkey coryza.

INTRODUCTION

Avian bordetellosis or turkey coryza is a highly contagious bacterial respiratory disease of different avian species, mainly turkeys (Ehsan et al., 2020). *Bordetella avium* (*B. avium*) bacterium is the causative agent of bordetellosis in domesticated turkeys (Skeeles and Arp, 1997; Boulianne et al., 2020) and chickens (Boulianne et al., 2020). Infection with *B. avium* is disseminated in domestic and wild birds (Stenzel et al., 2017). Since the 1970s, bordetellosis has been considered a major health problem in young birds especially turkey poults. The disease affects all ages of birds with a significant economic losses in poultry industry. Although bordetellosis causes low mortality, its high morbidity could be problematic for the production performance of broiler turkeys especially in early weeks of life. In the United States of America (USA), the disease is ranked as the third of the most important challenging diseases affecting turkeys industry (Beach et al., 2012) and causes several million dollars losses (Skeeles and Arp, 1997). Coinfections of turkeys with *B. avium* and other bacterial infections such as *Escherichia coli* (Van Alstine and Arp, 1987), *Ornithobacterium rhinotracheal* (El-Sukhon et al., 2002; Szabó et al., 2015), *Chlamydia psittaci*, *Pseudomonas fluorescens*, and *Klebsiella pneumoniae* (Hinz et al., 1992) were reported.

Bordetellosis was first described in Canada in 1967 (Filion et al., 1967). Simmons et al. (1976) also described bordetellosis as turkey rhinotracheitis. In Germany, Hinz et al. (1978) found a respiratory disease of turkeys as that previously observed in Canada and USA. Later on, bordetellosis has been widely distributed in many countries all over the world such as USA (Gentry-Weeks et al., 1991; Raffel et al., 2002; Beach et al., 2012), Australia (Blackall et al., 1995), Hungary (Szabó et al., 2015), Poland (Smialek et al., 2015; Stenzel et al., 2017), Brazil (Grespan et al. 2012), Turkey (Ozbey and Muz, 2006; Türkyilmaz et al., 2009), India (Balouria et al., 2019), Iran (Ehsan et al., 2020), Jordan (El-Sukhon et al., 2002), and Egypt (Abo-State et al., 2018; Erfan et al., 2018; Eldin et al., 2020). For instance, high prevalence rates of *B. avium* (54.54% and 49.18%) were detected in turkey flocks of Poland and Egypt, respectively (Stenzel et al., 2017; Eldin et al., 2020).

Accordingly, this article was designed to spotlight on avian bordetellosis regarding the bacterium pathogenesis, susceptibility, transmission, pathology, laboratory diagnosis, and prevention and control measures.

Citation: Abd El-Ghany WA (2022). Avian bordetellosis: a significant bacterial respiratory disease of turkeys (*Meleagris gallopavo*). *Online J. Anim. Feed Res.*, 12(3): 103-110. DOI: https://dx.doi.org/10.51227/ojafr.2022.14

The causative agent and pathogenesis

Previous preliminary studies designated the causative agent of turkey coryza as a *Bordetella*-like bacterium (Hinz et al., 1978). Later, this bacterium was known as *Alcaligenes faecalis* based on biochemical identification (Simmons et al., 1980). *Bordetella avium* is the causative of bordetellosis or turkey coryza. It is a bacterium belongs to the genus *Bordetella*, which contains 15 species. Moreover, *B. bronchiseptica*, *B. holmesii*, *B. parapertussis* and *B. pertussis* are present in mammalian hosts, but they are completely distant from *B. avium* that isolated from avian hosts. Other types of *Bordetellae* such as *B. ansorpii, B. bronchialis, B. flabilis, B. muralis, B. petrii, B. sputigena, B. trematum, B. tumbae* and *B. tumulicola* have not well studied.

In USA, the genome of 197N of *B. avium*, a spontaneous nalidixic acid-resistant variant of strain 197, was detected in a turkey (Gentry-Weeks et al., 1991), and then it was sequenced (Sebaihia et al., 2006). Besides, Ehsan et al. (2020) demonstrated that the sequences analysis of suspected culture in Iran were 98%, 96%, and 98% similar to *B. avium* 197N (AM167904.1), 4142 (AY925058.1), and 4156 (AY925068.1) sequences, respectively. Early in 70th, the preliminary studies designated the bacterial causative agent of coryzs in turkey as a *Bordetella*-like organism (Hinz et al., 1978). Simmons et al. (1980) biochemically identified *Bordetella*-like bacterium as *Alcaligenes faecalis*. Later, *B. avium* was defined as the causative agent of bordetellosis or turkey coryza disease. This bacterium was classified under the genus *Bordetella* that contains 15 species. Other *Bordetella* species such as *B. bronchiseptica*, *B. holmesii*, *B. parapertussis* and *B. pertussis* that were detected in mammalian hosts are completely distant from *B. avium* in avian hosts. Moreover, *B. ansorpii, B. bronchialis, B. flabilis, B. muralis, B. petrii, B. sputigena, B. trematum, B. tumbae* and *B. tumulicola* have not well studied in different avian species. The genome map of 197N of *B. avium*, a spontaneous nalidixic acid-resistant variant of strain 197, was detected in a turkey early in USA (Gentry-Weeks et al., 1991), and then the bacterium was full sequenced (Sebaihia et al., 2006). The recent Iranian study of Ehsan et al. (2020) showed that the sequences analysis of suspected culture were 98%, 96%, and 98% similar to *B. avium* 197N, 4142, and 4156 sequences, respectively.

Virulence key factors for *B. avium* pathogenesis can include specific adhesion to the respiratory epithelium cilia and local mucosal injury (Knab et al., 2020). *Bordetella avium* colonizes and adheres specifically to the local ciliated epithelium of the upper respiratory tract upon infection (Temple et al., 1998). Then, the bacterium induces ciliostasis, apoptosis, and extrusion of ciliated cells from the epithelium (Miyamoto et al., 2011). There was an association between the *in-vitro* ability of *B. avium* to adhere the tracheal ciliated epithelium and its ability to colonize the respiratory tracts of turkeys (Temple et al., 1998). *Bordetella* adhesion, represented by filamentous haemagglutinin, is present on the bacterial surface and plays an essential role in the adhesion and the colonization process of the host respiratory epithelium (Edwards et al., 2005). Therefore, fimbriae and hemagglutinin may contribute to *B. avium* pathological effects (Arp et al., 1988). Many *B. avium* isolates can produce toxins such as endotoxin, tracheal cytotoxin, heat-stable dermonecrotic toxin, and osteotoxin, besides hemagglutinine which are involved in the pathogenesis of bordetellosis (Figure 1; Rumińska and Koncicki, 1999). Gentry-Weeks et al. (1988) reported that both dermonecrotic toxin and tracheal cytotoxin are the main virulence factors for *B. avium in-vivo* pathogenicity as they concerned with the damage of tracheal tissue.

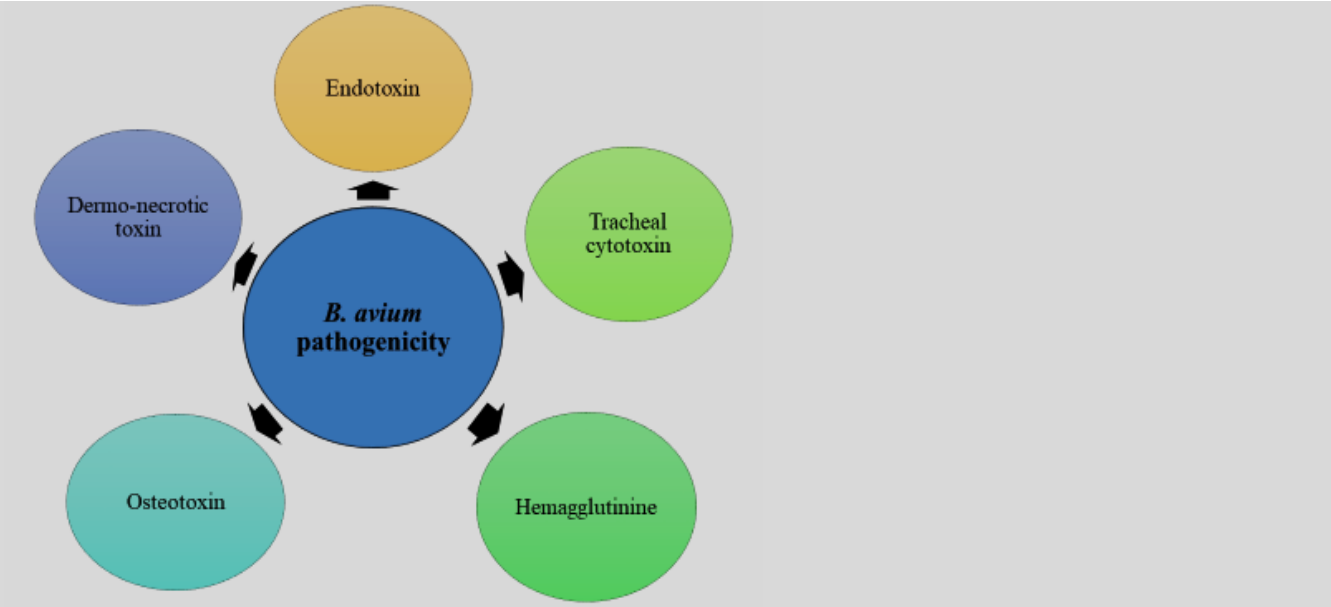

Figure 1 - Factors associated with the pathogenicity of *B. avium* infection

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Virulence-associated expression genes such as *bvgA*, *fhaB*, and *fimA* are crucial for the pathogenicity of *B. avium* (Spears et al., 2003; Temple et al., 2010; Linz et al., 2016; Eldin et al., 2020). The adhesion gen, *fhaB*, is very important and its expression is regulated by *bvgA.* Flagellar genes which are responsible for the motility and the attachment of *B. avium* to the epithelium are also essential for the organism virulence (Linz et al., 2016). Eldin et al. (2020) confirmed that *B. avium* strains carried virulence-associated genes including *Bordetella* virulence gene (100%), fimbriae (71.14%), and filamentous hemagglutinin (85.68%) which are responsible for colonization of the bacterium in the respiratory tract of turkeys. It has been observed that strains of *B. avium* from cockatiels and turkeys did not show difference in virulence-associated characters such as tracheal attachment or cytotoxic effects (Grespan et al., 2012). According to the results of Knab et al. (2020), both adherence and ciliostasis assays could be used for characterization of *B. avium* virulence and any reduction of virulence could be attributed to the variations in the filamentous haemagglutinin protein. Hemagglutinin produced by *B. avium*, besides autotransporter proteins and fimbriae play the major role adhesion process (Loker et al., 2011; Stockwell et al., 2011). It has been found that *B. avium* can agglutinate guinea pig erythrocytes and the loss of hemagglutination capability results in bacterial attenuation (Temple et al., 2010).

Susceptibility

Species

A wide range of avian species develop bordetellosis, but turkey is regarded as the most susceptible host. Domestic and wild birds are susceptible to the disease (Raffel et al., 2002). In Germany, *B. avium* was isolated from domestic and wild birds such as Muscovy ducks, geese, a yellow-crested cockatoo, parrot finches, and partridges. Moreover, *B. avium* was molecularly detected in the tracheal swabs of common pheasants in Poland (Stenzel et al., 2017). Infection with *B. avium* causes locked jaw syndrome in cockatiels (Grespan et al., 2012). High titers of antibodies against *B. avium* were detected in Canada goose, but relative low titers were present in pigeons and doves (Raffel et al., 2002).

Age

All ages of birds can get *B. avium* infection, however, younger ages are more susceptible than adult (Hinz et al., 1978; Kersters et al., 1984). Two to 6-week-old turkey poults are highly susceptible to infection. Smietanka et al. (2014) found that 3-week-old turkey poults are likely to be infected sub-clinically with *B. avium*. Significant positive association between the age of birds and the titer of anti-*B. avium* antibodies was observed. Previous exposure to *B. avium* leads to development of antibodies which might be transferred to the hatching poults causing reduction of the clinical signs severity. Moreover, an increase in the titer of antibodies to *B. avium* was detected by increasing the age of turkeys from 5 to 56 days (Beach et al., 2012). Adult birds had *B. avium* infection at young age showed detectable level of antibodies in response to that infection, while young birds have had less time to become infected and develop antibodies (Raffel et al., 2002).

Infection and transmission

Horizontal transmission is important for the spread of *B. avium* infection. Transmission may occur via aerosol, water, or litter contamination, and the bacterium can remain viable and virulent in humid litter for at least 6 months (Skeeles and Arp, 1997). The bacterium also seems to live in water like other *Bordetella* species (Porter and Wardlaw, 1993). Additionally, presence of *B. avium* in solitary species such as wood thrush indicated that reservoirs may be important for the direct contact among birds in the wild (Raffel et al., 2002). Therefore, transmission of *B. avium* from free-living birds to domesticated poultry may be possible. *Bordetella avium* does not transmitted vertically from dams to their offspring (Jackwood and Saif, 2008). Exposure of birds to different management environmental stress conditions is an important predisposing factor for spreading of infection (Bartz et al., 2018). This indicates the role of biosecurity measures in the control of turkey coryza. Moreover, it has been reported that immunosuppressive viral infections could enhance the severity of *B. avium* infection (Liang et al., 2013).

The similarities between *B. avium* and *B. pertussist*; one of the causes of human's respiratory affections have been demonstrated (Gentry-Weeks et al., 1988; Spears et al., 2003). Lastly, *B. avium* has been detected in persons with cystic fibrosis (Spilker et al., 2008). Accordingly, both *B. avium* and *B. avium*-like organisms are regarded as opportunistic pathogens for humans. The gene sequence analysis of 16S rRNA of *B. avium* and *B. pertussist* revealed 98% and 100% nucleotide similarities with *B. avium* ATCC 35086 strain (Harrington et al., 2009).

Pathology

Bordetella infection in poultry is characterized by sudden onset and rapid spread. The severity of clinical signs is milder in chickens than in turkey poults. Respiratory signs including sneezing, foamy nasal and ocular discharge, submaxillary edema, cough, moist tracheal rales, dyspnea, and altered vocalization could be observed in susceptible birds (Jackwood and Saif, 2013). The disease course is about 2–4 weeks (Panigrahy et al., 1981). Bordetellosis is characterized by high morbidity and low mortality rate especially in cases without complications. Turkeys had passive humoral immunity

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against *B. avium* were protected against the development of clinical signs after experimental infection (Hinz et al., 1981; Rimler and Kunkle, 1997). Postmortem lesions of *B. avium* infection include conjunctivitis, presence of exudates in the nasal cavity, trachea, and bronchi, pneumonia, and cloudiness and turbidity of air sacs (Saif et al., 1981; Arp and Cheville, 1984). Deformity of tracheal rings and damage to articular cartilages (collapsed trachea) may be related to osteotoxin activity (Stenzel et al., 2017). Complicated cases with secondary bacterial and viral infections showed visceral lesions. Tracheal sections of *B. avium* infected turkeys showed mucosal separation, lympho-plasmacytic infiltration, and loss of cilia (Van Alstine and Arp, 1988). Besides, severe pneumonia with congested blood vessels, and airsacculitis associated with edema and infiltration of lymphocytes and macrophages were also observed (Eldin et al., 2020).

Laboratory diagnosis

Isolation

Culturing of *B. avium* was previously described (Register and Jackwood, 2016). The bacterium grows aerobically on blood agar plates supplemented with 10% sheep blood for 24–48 hrs. Other concomitant fast growing bacteria such as *Escherichia coli* may associated with *B. avium* infection that can cover the growth of *B. avium* and may create some difficulties in the isolation process. Therefore, some antibiotics such as aztreonam and ampicillin could be added to the culture media to inhibit the growth of other opportunistic bacteria without affecting *B. avium* growth. It has been detected that administration of antibiotics before sampling may induce failure of *B. avium* isolation (Türkyilmaz et al., 2009).

Identification

Typical colonies of *B. avium* isolates are small or pinpoint, glistening, translucent, compact, and pearl-like with glistening surface with entire edges. Colonies of *B. avium* are 1–2 mm in diameter after 48 hs of incubation. Gram staining of suspected culture revealed Gram-negative bacilli. Biochemically, *B. avium* shows positive reactions for catalase, oxidase, and citrate, but negative reaction for lactose fermentation, urease, and nitrate reduction (Simmons et al., 1980). Moreover, on triple sugar iron agar, no acid production in the butt or the slant was determined after overnight incubation of *B. avium* isolates, however, H_2S production was detected. Biochemical tests were also used to distinguish *B. avium* from other non-fermentative bacteria or other *Bordetella* species such as *B. hinzii* (Blackall and Farrah, 1986).

Molecular characterization

Molecular assays such as *Pvu*II ribotyping and restriction endonuclease analysis using either *Hinf*I or *Dde*I could be used to distinguish *B. avium* from other species of *Bordetellae* (Sacco et al., 2000). Polymerase Chain Reaction (PCR) is also a valuable tool to detect *B. avium* infection (Ehsan et al., 2020). This test showed 100% sensitivity and 98.8% specificity for the identification of *B. avium* from different locations over 25 years (Register and Yersin, 2005). TaqMan real-time PCR detected presence of *B. avium* in tracheal swabs of pheasants at 54.54% in Poland (Stenzel et al., 2017). In the Egyptian study of Eldin et al. (2020), the overall PCR-confirmed prevalence rate of B. *avium* was 22.95% (14 out of 61). Although antibodies against *B. avium* could be serologically detected in the serum of turkeys, the PCR test may indicate negative results (Türkyilmaz et al., 2009). For the first time in Egypt, Erfan et al. (2018) reported on sequencing of *B. avium* isolates from turkeys and found that amplified 740 bp of *rec*A gene showed 100% maximum identity with German *B. avium* ATCC 35086 strain and with the American strain 197N.

Enzyme Linked Immuno-sorbent Assay

It has been shown that Enzyme Linked Immuno-sorbent Assay (ELISA) is considered as the most sensitive test for serological screening of *B. avium* infection in turkeys and chickens (Tsai and Saif, 1991). This test can give an indication for the previous or recent exposure of the flock to *B. avium* infection. The prevalence rate of *B. avium* among turkey flocks in Egypt was 72.13% (44 out of 61) using ELISA (Eldin et al., 2020). In Poland, Smialek et al. (2015) found anti-*B. avium* immunoglobulin Y in young and old ages turkeys. A positive correlation between the increase in the antibody titers against *B. avium* and the age of the birds was detected (Stenzel et al., 2017). Past exposure of turkeys to *B. avium* infection resulted in development of passive humoral immunity and protection of birds from the development of signs after experimental infection (Rimler and Kunkle, 1997). Additionally, Beach et al. (2012) found an increase in the titer of *B. avium* antibodies by increasing the ages of turkeys. Positive significant correlation has been reported between the age of diseased turkeys and the titer of antibodies against the bacterium (Eldin et al., 2020).

Prevention and control measures

Biosecurity measures

Application of strict biosecurity measures in turkey flocks is the must for prevention of bordetellosis. Thorough cleaning and disinfection of farms are crucial as the bacterium can persist for months in damp litter (Van Alstine, 1987). Thus, clean up measures are required to remove *B. avium* from contaminated premises, besides hygienic disposal of litter, and disinfection of all surfaces, feeders, and drinking water systems.

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Treatment

Antibiotic treatment of *B. avium* shows variable success; it is likely that antibiotics may treat the secondary bacterial infections instead of *B. avium*. Very early study of Glunder et al. (1979) showed that water treatment of *B. avium* infected turkeys with sulphaquinoxalin/trimethoprim or tetracycline for 5 days was effective, but relapse occurred after the discontinuation of the treatment and the carrier birds were not eliminated. Thus, treatment of *B. avium* with various drugs was often ineffective. Isolates of *B. avium* showed variations in their susceptibilities to various antimicrobials in different countries (Beach et al., 2012; Grespan et al., 2012). Formerly, strains of *B. avium* showed tetracycline resistance (Cutter and Luginbuhl, 1991). Further, in Minnesota in 1998–1999, 4 isolates of *B. avium* were susceptible to ampicillin and tetracycline, while they were resistant to erythromycin (Malik et al., 2005). Moreover, 17 *B. avium* strains collected over thirty years from turkey flocks in USA revealed sensitivity to gentamicin, cefoperazone, cefepime, ceftazidime, piperacillin, and amikacin, and resistance to chloramphenicol, ampicillin, cipfloxacin, sulfa-trimethoprim, and oxytetracycline (Beach et al., 2012). In Hungary, turkey isolates of *B. avium* showed complete resistance to ceftiofur and lincomycin, and moderate resistance to chloramphenicol and nalidixic acid, but complete sensitivity to sulfa-trimethoprim, polymyxin B, and gentamicin (Szabó et al., 2015). Among 50 *B. avium* isolates, the range of resistance against cephalosporin, penicillin, erythromycin, and enrofloxacin were more than 50% (Nhung et al., 2017). Ehsan et al. (2020) detected that *B. avium* isolates in Iran were partially sensitive to ampicillin. The author's referred ampicillin-resistance of *B. avium* isolates to the lack of penicillin-binding protein 3 (PBP3) gene. In an Egyptian study of Erfan et al. (2018), the results of the antibiogram showed that *B. avium* isolates were susceptible to norfloxacin, ciprofloxacin, cefotaxime, florfenicol, and gentamicin, while isolates were resistant to ampicillin, erythromycin, oxytetracycline, sulphamethxazole/trimethoprime, and lincomycin exhibited the highest resistance rates. Moreover, Egyptian isolates of *B. avium* were resistant to penicillin, ceftiofur, nalidixic acid, and lincomycin, but sensitive to gentamicin and neomycin (Eldin et al., 2020). These differences in sensitivities or resistances among *B. avium* isolates might be owing to the uncontrolled application of antimicrobials and presence other concurrent infections and environmental factors.

In a study of Ehsan et al. (2020), in Iran, the author referred the partial ampicillin sensitivity to the absence of penicillin-binding protein 3 gene in *B. avium* strains. Some Egyptian *in vitro* studies revealed that isolates of *B. avium* were resistant to ampicillin, lincomycin, erythromycin, sulphamethxazole/trimethoprime, and oxytetracycline, but they were sensitive to ciprofloxacin, norfloxacin, gentamicin, florfenicol, and cefotaxime (Erfan et al., 2018). Nearly similar Egyptian study of Eldin et al. (2020) demonstrated that *B. avium* were resistant to penicillin, lincomycin, ceftiofur, nalidixic acid, while they were susceptible to gentamicin and neomycin.

Vaccination

Vaccination of turkeys is another approach for prevention of bordetellosis. Commercially available vaccines for bordetellosis may have marginal efficacy, possibly due to vaccine delivery or strain specificity. As a result of *B. avium* persistence in the premises of the infected farm, vaccinated poults would experience an anamnestic response due to continuous exposure to infection.

Living vaccines against bordetellosis have been used in many countries especially in the early life of turkey. Therefore, detection of maternal derived antibodies is important prior to vaccination (Smialek et al., 2015). Oral vaccination with a temperature-sensitive mutant strains of *Alcaligenes faecalis* could protect turkey flocks and produced humoral antibodies in vaccinated turkeys without development of alcaligenes rhinotracheitis outbreaks (Jensen and Marshall, 1981). Moreover, Burke and Jensen (1981) demonstrated that vaccination of 6-week-old turkeys with a temperature-sensitive mutant of *Alcaligenes faecalis* via drinking water in doses of 90 million bacteria resulted in development of a high degree of protection against challenge with the same bacterium.

Twice vaccinations of turkey poults with *B. avium* inactivated oil-adjuvant vaccine at 4 and 27 days of age induced significant immune response to the challenge at 41 days of age and the vaccinated birds were able to eliminate the bacteria faster than non-vaccinated ones (Glunder et al., 1980). Jackwood and Saif (1980) did not find maternal immunity against the infection in offspring's after vaccination of turkey parent hens with formalin inactivated bacteria or with an aluminium hydroxide adjuvant bacterin. Whereas, the study of Hinz et al. (1981) showed that subcutaneous vaccination of 24 and 28-week-old turkey breeder flocks with heat-inactivated and Freund's adjuvant *B. avium* bacterin could protect the progeny against infection within the first 10 to 17 days after hatching. Moreover, agglutinating antibodies were transmitted via the yolk and detected in the progeny. Johnson et al. (1980) found that convalescent turkey flocks with a history of previous exposure to *Bordetella* infection showed usual delay in the onset as well as the severity of the disease. Progeny from vaccinated breeder turkey hens with double doses of oil emulsion adjuvanted *Alcaligenes faecalis* bacterin showed considerable resistance to infection, improved livability and growth, and delayed onset of infection with less severe clinical picture even after infection (Barnes and Hofstad, 1983). Subcutaneous vaccination of day-old turkey poults with formaldehyde and aluminum hydroxide *B. avium* inactivated bacterin induced antibody titers twice the levels that were found in non-exposed flocks. Moreover, Akeila and Saif (1988) studied the effect of oil-adjuvant pili in protection against *B. avium* infection in turkey poults in comparison with other types of bacterins. The authors postulated that *B. avium* pili are important immunogens in terms of reduction of the disease severity and isolation of the bacterium from the respiratory tract.

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CONCLUSION

Avian bordetellosis represents a significant problem especially for turkey industry. Research work regarding the surveillance studies of *B. avium*, especially in some developing countries, is much needed. In addition, increasing awareness about the rational usage of antimicrobials will decrease the possibilities of *B. avium* treatment failure, and consequently reduce related financial losses. Trials for preparation of local or autogenous vaccine against *B. avium* is crucial as a preventive measure. Besides, application of strict biosecurity measures can reduce the incidence of bordetellosis among turkey flocks.

DECLARATIONS

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Author's contribution

Abd El-Ghany WA has collected and drafted the manuscript, formatted it, and approved the final manuscript.

Conflict of interests

The author has not declared any conflict of interest.

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ZOO-TECHNICAL PERFORMANCE OF INDIGENOUS DAIRY COWS UNDER SMALL HOLDER FARMERS MANAGEMENT SYSTEM IN HAWELLA-TULLA DISTRICT, ETHIOPIA

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Supporting Information

ABSTRACT: Ethiopia's dairy production system is predominantly extensive, with poor cattle performance constrained by a variety of factors such as poor genetics, low reproductive performance, and poor quality of feed, among others. So far, little work aimed at increasing dairy production has been undertaken. The objective of the study was to characterize the zoo-technical performance of indigenous dairy cows under small holder farmer management system. The study was undertaken in Hawella-Tulla district, Sidama Region; Sothern Ethiopia from November 2020 to March 2021. From three peasant associations (PA), 180 smallholder dairy farmers who owned local cows were selected using a simple random selection technique. Data were collected using a structured questionnaire. The majority of farmers (75%) practiced in mixed crop-livestock farming primarily produce milk for income generation. Farmers hold an average of 1.95±0.063 local lactating cows. The productive performance of the indigenous cow mean of daily milk yield was 1.53±0.09 liters and 6.5±0.89 months of lactation length. The mean age at first service (AFS) was 38.5±2.71 months, Age at first calving (AFC) was 45.3±2.82 months, calving interval (CI) was 20.08±0.9 months, and number of services per conception (NSPC) was 2.5±0.63. It can be concluded that the zoo-technical performance of local cows was very low. Therefore, planned technical and institutional intervention to improve the feeding system, provide better health management, genetic improvement of local breeds through crossbreeding and synchronization should be carried out for the betterment performance of local cows.

RESEARCH ARTICLE

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INTRODUCTION

Dairy production is an essential part of livestock farming in Ethiopia (Getabalew et al., 2019). Dairying, however, has not been completely utilized and stimulated (Sintayehu et al., 2008; Minten et al., 2020). Moreover, due to a lack of infrastructure and market links, commercialized smallholder dairy production is not effectively practiced, with only around 15% of the output reaching the market. As a result, the dairy sector's contribution to the country's agricultural economy remains lower than it could be (FAO, 2017).

Ethiopia's milk production is growing slowly. The annual milk production growth rate of 1.2 percent lags behind the expected yearly human population growth of 3%. About 97 % of annual milk production of the countries is produced under the smallholder milk production system, which is dominated by local breeds with low production levels (Getabalew et al., 2019). The Zoo technical performance of the herd or animal is a key indicator of the sustainability of a dairy farming system. In this regard, the performance record of local cows is essential for designing breeding as well as management strategies in dairy sector (Abrha et al., 2020). Zoo technical features such as age at first service, calving interval, number of services per conception, average milk yield per day, and calving interval determine cow reproductive and productive efficiency (Neto et al., 2018). It is further noted that these traits are critical in terms of the economics of dairy management (Abrha et al., 2020).

The Hawella-Tulla district is one of the potential dairy cattle production areas, which is dominated by indigenous dairy cows. Despite this, the performance of dairy cows has not been scientifically documented. So far, little research has been conducted to identify the overall productive and reproductive performances of indigenous dairy cows managed by smallholder farmers. So, an understanding of Zoo-technical performance of indigenous cows under smallholder farmer's management system provides guidance as to which of the possible new technologies are appropriate and worth pursuing in order to increase productivity in indigenous dairy cows. Thus, studying the zoo-technical performances of local cows is crucial in order to generate baseline data that livestock owners, extension agents, and researchers can use to develop improvement and development strategies for local cow productivity. On the basis of this, the current study investigated the zoo-technical performance of indigenous cows in the Hawella–Tulla district under a smallholder management system.

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MATERIALS AND METHODS

Description of the study area

This research was carried out in the Hawella-Tulla district of Sidama Regional State, which is 10 kilometers from Hawassa City and 285 km south of Addis Ababa. Geographically, it is located between 6.45 degree and 38.7 degree longitude east, and between 6.33 degree and 6.62 degree latitude north. The district is one of the eight districts in the Hawassa city administration. The district has a total population of 138,979. The average annual rainfall is 1124 mm. The average altitude is 1710 meters, with a maximum temperature of 32 degrees Celsius and a minimum temperature of 28 degrees Celsius. Maize is the dominant crop grown in the area. Cattles are the most common livestock in the area (Teklemariam and Cochrane, 2021).

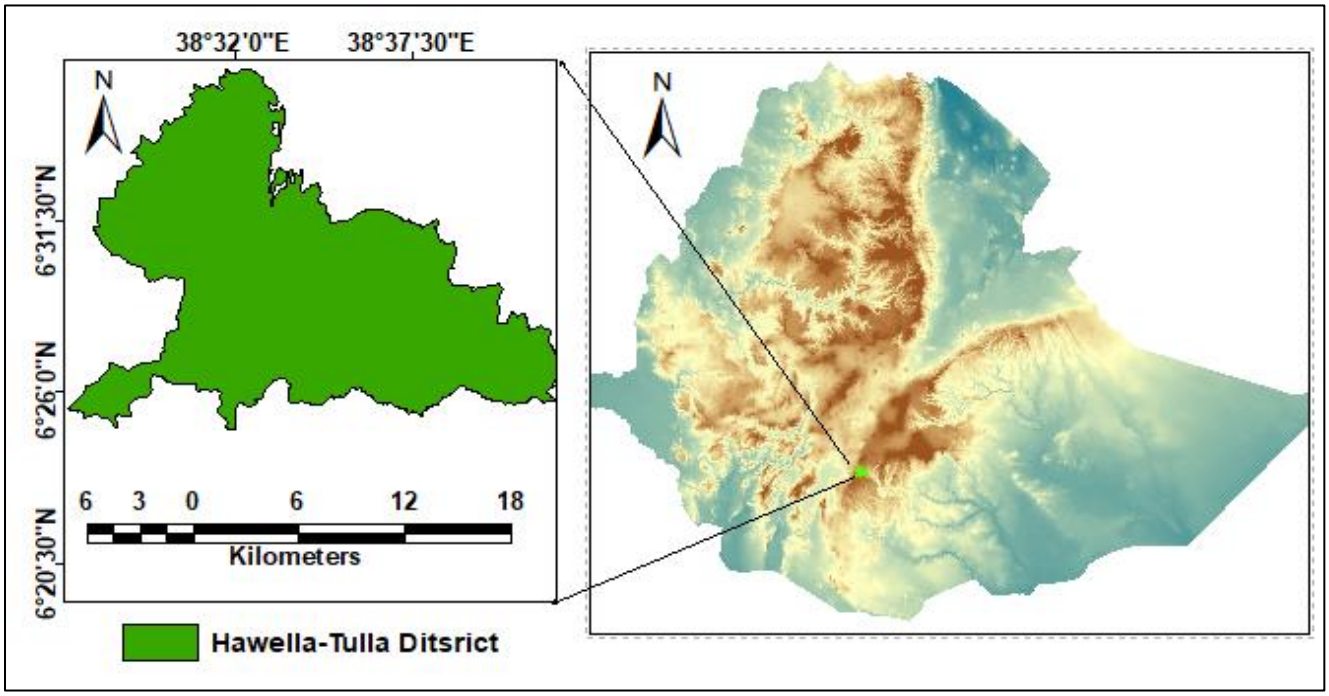

Figure 1 - Location map of Hawella-Tulla District

Sampling procedures/ techniques

The survey was conducted during the months of November 2020 to March 2021. A reconnaissance survey instrument was developed and conducted in order to select specific dairy farmers and to get a general picture of the study sites. A two-stage purposive and random sampling technique was implemented to select research units. First, after taking information on the production potentials of all kebeles from the district, three kebeles (peasant associations) were selected purposively based on their potential in dairy cattle production and minimum holding of at least one local cow. Second, a total of 180 smallholder dairy farmers from three kebeles were selected using a simple random selection technique and the sample size determination formula 1 presented by Yamane (1965).

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

Where, n is the sample size, N is the population size and e is the level of precession. The total dairy cattle producers in the district were 301 and 4.75% precision was used

$$n=\frac{301}{1+301(0.475)^2} = 179.2 \text{.....2}$$

As a result, an approximation was employed, and the total number of small holder dairy producers used was 180.

Sources and methods of data collection

A cross-sectional survey was undertaken on 180 purposely selected smallholder dairy farmers from three potential kebeles (Cheffe, Dato and Tulla). Primary data was collected through a pretested structured questionnaire. Secondary data were collected from documents of district's livestock and fisheries office and other related articles.

Data analysis

The data was encoded and cleared using Microsoft Office Excel 2019 and descriptive statics were generated after analysis using SPSS (statistical package for social science, version 26). Percentages, means tables, and standard error

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were used to illustrate the summarized results. A person chi-square test was calculated using a statistical analysis system to see whether or not the proportions of the variables differed significantly.

Ethical clearance

The data collection tool (field survey questionnaire) were assessed and approved by Dilla University's College of Agriculture and Natural Resources Furthermore, all of the surveyed smallholder farmers in this study provided informed consent.

RESULTS AND DISCUSSION

Characterization of production system

The significant difference in characterization and purpose of production system of respondents was tested at 5% probability. According to the respondents, 75% practiced mixed crop-livestock farming, indicating a significant difference ($P<0.05$) in the percentage of farmers that practiced mixed production compared livestock production (Table 1). The findings are comparable to those of Asrat et al. (2016) in Wolaita Sodo Town, Southern Ethiopia. This implies Crop cultivation and cattle production are complementary activities for the majority of the respondents. Cereal crop based system (Maize) is the major system under the mixed crop-livestock production system in the Hawella-Tulla district. A significant number of smallholder dairy farmers (58.3%) were producing milk primarily for income generation. Getabalew et al. (2019) also mentioned milk production as income generation/sale purpose in small scale dairy production system.

Characterization of local cattle herd compositions

The average herd composition of the assessed small holder dairy farms in the district is summarized in Table 2. Farmers hold an average of 1.95±0.063 local lactating cows. In Dilla-Zuriya district, Hailemariam et al. (2022) mentioned that smallholder farmers hold of 2.92 ± 1.18 local cows. The study identifies that even though indigenous cow are low milk producer, and they were the major source of milk in the study area. According to Hailemariam et al. (2022), keeping the most cows may be beneficial due to their various functional uses in milk production, replacement stock, and manure. Farmers, on the other hand, rarely kept local bulls. This lower average of bulls in the current study could be attributable to a major land limitation on providing adequate feed for their animals.

Zoo-technical performance of local cows

Daily milk yield and lactation length

The mean daily milk yield (DMY) of local cow under small holder farmer management system was 1.53±0.09 liters. The DMY recorded in this study was comparable with the report by Yetera et al. (2018) who reported 1.51± 0.08 liters in selected parts of Sidama Zone, but lower than that reported Abdurehman Musa and Yusuf Mummed (2020) which was 1.94 ±0.154 liters in West Hararghe, Oromiya Regional State and 1.8±0.045 liters in Dawero zone by Hussein (2018).

The difference in daily milk yield could be explained by feed availability, animal health management, agro-ecological zones difference, and variations in farmers' practices of keeping selected local cows. In terms of nutrition, for example, the research conducted by Mekuriaw et al. (2020) in Fogera local cow showed that the milk yield in the cows fed Brachiaria hybrid grass hay was almost double (52.97%) that of the natural pasture hay diet. These results suggest that better feeding and fodder incorporation could increase milk yield in indigenous dairy cows.

The lactation length (LL) is a crucial production trait since it affects total milk yield (Vijayakumar et al., 2017; Demeke, 2020). The lactation length found in the Hawella-Tulla district was 6.5±0.89 months. The current study's mean LL of local cow under farmer's management was lower than the reports of Kibru et al. (2015), Abrha et al. (2020), Adane and Ayalew (2020), and Demeke (2020) in Central Tigray, Chuko, Gondar, Angot District respectively. The LL in this study was also lower than the national average of seven months (Mekuriaw and Harris, 2021). This indicates that cows in the current study area had shorter lactation periods. This could be due to feed as well as other management issues such as breed improvement practices. As a result, addressing these issues will improve the cow's LL. For example, a study by Ayalew and Asefa (2013) indicated that better feeding conditions and cross breeding improved the lactation length of crossbred Holstein Friesian–local cows to 11.13 ± 4.84 months compared to the local cow LL of 9.13 ± 2.6 months. Most Ethiopian local cows have a short lactation period, indicating that they have been kept for an extended length of time without generating any output (milk or calf), instead relying on feed and other production costs.

Reproductive performance of local cows

Age at first service (AFS)

The mean age at first service (AFS) was found to be 38.5 ± 2.71 months (Table-3). The current finding is lower than AFS of 44.1±5.9 months reported by Belay (2016) and 42.61±2.82 by Yetera et al. (2018) for local dairy cows in Sidama Zone, Southern Ethiopia. In Siltie Zone, Tolasa (2021) mentioned lower AFS (37.5± 13.5). This variance could be attributable to various factors such as feed availability and access to concentrate feed, artificial insemination availability, and dairy husbandry practices. Farmers remarked that the likely cause of the delayed first service is slow heifer growth, which is largely influenced by feed and animal health management.

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Age at first calving (AFC)

Age at first calving , or the time it takes a female calf to achieve puberty and reproduce for the first time, is a significant element in the expense of rearing replacements in dairy herds (Atashi et al., 2021). Local cows in Hawella-Tulla district had a mean age at first calving of 45.3±2.82, which was comparable to the AFC of 45.13±2.31 in Angot District reported by Demeke (2020). It was, however, greater than the mean age at first calving (AFC) in Central Tigray, which was 43.3±2.7 months by Abrha et al. (2020) and lower than the AFC of 52.30±2.73 months reported by Yetera et al. (2018). These variations could be linked to a shortage of feed sources, as environmental factors, notably nutrition, can affect heifer growth and maturity, as well as the normal development of reproductive organs in order for them to perform their functions. Farmers noted that a cow's nutritional state has a huge impact on AFC.

Calving interval

The calving interval is the period time between two consecutive parturitions (Atashi et al., 2021). The mean calving interval (CI) in this study was 20.08±0.9 months, which was greater than the CI of 16.02±0.29 months reported in West Hararghe, Oromiya Regional State by Abdurehman Musa and Yusuf Mummed (2020) and with CI of 19.93 ±0.18 by Kibru et al. (2015). However, the current finding was comparable with the CI of 20.08±0.90 by Yetera et al. (2018) and lower than CI of 23.6±4.4 by Belay (2016). The variation could be linked to feed shortages, the calving season, health care, and a lack of fodder availability in indigenous dairy cows documented across the country. Farmers in the study area believe that silent estrus and a lack of adequate heat detection are important reasons in the delayed CI of local cows.

Number of services per conception (NSPC)

In the Hawella-Tula district, the average number of services per conception of indigenous cows was 2.5±0.63. The mean NSPC is greater than the NSPC of 1.81 for indigenous cows in Silte zone (Tolasa, 2021). Furthermore in smallholder farm situations in and around Maksegnit Town NSPC of 2 was reported by Alemayehu and Moges (2014) and 1.8 by Belay (2016) in Sidama zone. According to Kumar et al. (2014), indigenous cows in Gondar had a considerably higher NSPC (2.2±0.2) than HF crossbreds (1.5±0.3) under a small holder management system. According to Mukasa-Mugerwa (1989), a number of services per conception greater than two should be considered poor. Poor reproductive management, a lack of effective heat detection, and a lack of timely insemination could all be contributing factors to the study area's high NSPC. NSPC depends on the breeding system used. It is higher under uncontrolled natural breeding than artificial insemination. Moreover Silent estrus also increases the NSPC of local cows in the study area.

Table 1- Characterization production system dairy in Hawella-Tulla district

Description		Cheffe N (%)	Dato N (%)	Tullo N (%)	Overall %	X2	P-value
Production system	Livestock production	12(20)	18(30)	15(25)	25	37.545*	0.0001
	Mixed crop-livestock farming	48(80)	42(70)	45(75)	75		
Purpose of milk production	Income generation/sale	18(30)	48(80)	39(65)	58.3	18.045*	0.001
	Home consumption	12(20)	3(5)	6(10)	11.7		
	Both	30(50)	9(15)	15(25)	30		

χ2 =Chi-square; * = significant if p < 0.05 level of significance.

Table 2 - Local cattle herd structure in Hawella-Tulla District

Local cattle herd structure	Mean ± SE
Lactating cows	1.95±0.063
Dry cows	1±0.001
Heifer	1.17±0.167
Calves	1.06±0.062
Bull	0.25±0.02

Table 3- Zoo- technical performance of local cows in Hawella-Tulla District

Parameters	Mean ±SE
Daily milk yield (liters)	1.53±0.09
Lactation length (month)	6.5±0.89
Age at first service (month)	38.5±2.71
Age at first calving (month)	45.3±2.82
Calving interval (month)	20.08±0.9
No. of service per conception	2.5±0.63

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CONCLUSION

Smallholder dairy farmers in Hawella-Tulla district produce milk under a mixed crop-livestock production system primarily for income generation. Local cows managed by small-holder farmers had low daily milk yields, short lactation lengths, lengthy calving intervals, and a large number of services per consumption. Based on the current survey results, it can be stated that the zoo-technical performance of local cows was very poor. This requires coordinated technical and institutional interventions to improve the feeding system, provide improved health management, genetic development of indigenous breeds through cross breeding, and estrus synchronization. Smallholders in the study areas should be encouraged to improve and expand their crossbred dairy cattle production.

DECLARATIONS

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Authors' contribution
I contribute with idea generation, data collecting, and analysis, as well as manuscript writing.

Conflict of interests
The author declares no conflict of interest.

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EVALUATION OF AMELIORATIVE EFFECTS OF MATURE COCONUT WATER SUPPLEMENTS ON *Cyrtosperma merkusii* ROOT MEAL INCLUDED DIETS FOR BROILER CHICKENS

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Supporting Information

RESEARCH ARTICLE

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ABSTRACT: The simultaneous degradation of essential nutrients is the major drawback in detoxifying anti-nutrient toxic substances of root meal-based diets. An ameliorative dietary supplement for root meal-based diets without undergoing thorough detoxification is proposed. Therefore, this trial aims to determine the effects of mature coconut water (MCW) against the anti-nutritional factors (ANF)-containing *C. merkusii* root meal diet on growth performance, carcass characteristics, and organoleptic traits of broiler chickens. Cobb 500 (*n*=160) day-old (male) grouped into the standard diet (commercial maize-soybean) or ANF (15% raw *C. merkusii* + 85% commercial maize-soybean) diet group, and the birds every group further allocated into 0, 5, 10, and 15% MCW water treatment groups (*n*=5) with four replications. The trial lasted for 20 D (8 to 28 D of age). In the ANF diet, treatment with MCW significantly differs on feed conversion ratio (FCR). The 5-15% MCW treated chickens were more feed-efficient than the 0% MCW treatment. MCW treatments were not significant on body weight, weight gain, survival, carcass component, and organoleptic traits of broiler chickens under the standard or the ANF diets. However, significant diet*water interactions were observed on BW and dress weight, and significant gizzard weight due to diets. In general, the improved FCR may be the ameliorative effect of mature coconut water against ANF on raw *C. merkusii* root meal inclusion (15% + 85%) in the standard diets.

Keywords: Ameliorative effect, Anti-nutritional factor, *Cocos nucifera*, *Cyrtosperma merkusii,* Root meal.

INTRODUCTION

In livestock farming, reduced production costs without compromising the welfare and performance, quality of meat and meat products is the primary concern (Bonnet et al., 2020; Post et al., 2020). The primary feedstuffs used for providing energy and protein in commercial poultry diets are maize and soybean (Maisonnier-Grenier et al., 2004; Hussein et al., 2020) because of their high digestibility. Several ingredients were experimented with to replace maize or soybeans, but none was proven effective in nutrient composition and cost-effectiveness (Medugu et al., 2011; Uguru et al., 2022). *Cyrtosperma merkusii* tuber, on the other hand, showed economic and phytonutrient potential over the maize but is instead characterized; by the low density of nutrients (Temesgen and Retta, 2015; Temesgen et al., 2017), causing feed stress due to anti-nutritional factors (ANF) which are considered toxic (Kumar, 1992; Samtiya et al., 2020; Taer et al., 2022). Anti-nutrient substances interfere with average growth, reproduction, and health when consumed regularly and are considered harmful and toxic (Bora, 2014). To safely consume the root and tuber products, detoxify them directly by heating them beyond 150°C, breaking the tubers to allow more substantial contact, microbial detoxification, and combining the techniques above (Okereke, 2012). However, the simultaneous degradation of essential nutrients is the major drawback during the detoxification process (Latif and Müller, 2015; Araújo et al., 2017). Infusing supplements in addition to root meal-based diets rather than thorough drying at high temperatures and detoxification is an interesting intervention. In a recent study conducted on broiler chickens supplemented with fresh coconut milk (FCM) under *C. merkusii* root meal diets, the result showed that giant swamp taro *C. merkusii* can replace maize up to 25%, giving a better body weight, weight gain, and feed conversion ratio compared to corn-soya-based diets (Taer et al., 2022).

The coconut water from the endosperm of the coconut (*Cocos nucifera* L.) recently had increased demand in beverage industries worldwide due to its flavoring, nutritional and therapeutic potential. Mature coconut water is the by-product left over by many coconut industries that produce virgin coconut oil, coconut milk, and desiccated coconut (Vani et al., 2021). The majority of the coconut mills discharge mature coconut water of around 261 MT volume per year (Prades et al., 2012). In India, approximately 80% of the mature coconuts gone processed into the desiccated coconut, also known as copra (Vani et al., 2021). In the Philippines, the coconuts are mostly processed on-field (on coconut farms), leaving coconut water wasted and unutilized. Thus, this massive volume of mature coconut water leftover promotes environmental concerns. For this reason, various thermal, non-thermal processing, and preservation methods extend the shelf life of coconut water (Naik et al., 2020) to meet the global market demand and a strategy of utilizing the liquid waste discharge (Vani et al., 2021). However, the applicability of this processing and preservation strategy is limited to

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coconut processing plants. The widespread of the rural coconut field and the scarcity of farm-to-market roads restrict the collection and transport of coconut water wastes from the coconut fields to the processing plant.

Coconut water (CW) is a natural, nutrient-rich, and refreshing drink in Asia, Europe, North America, Australia, and other countries. In folklore medicine, coconut water is drunk for oral rehydration and treatment of childhood diarrhea, gastroenteritis, and cholera and is also known to possess antioxidant properties (Mandal et al., 2009; Elekwa et al., 2021). The efficacy of plant materials in the treatment of chemical toxicity is associated with the presence of phytochemicals and other nutrients and bioactive compounds, which are known to have antioxidant properties. Coconut water has a therapeutic effect, containing various nutrients such as minerals, vitamins, antioxidants, amino acids, enzymes, and growth hormones (Bhagya et al., 2012; Halim et al., 2018; Johnkennedy et al., 2013; Zulaikhah, 2019). Recent studies on CW showed that it contains L-arginine, a free-form amino acid, and vitamin C, which can prevent heart disease and lipid peroxidation (Bhagya et al., 2012; Prathapan and Rajamohan, 2011). L-arginine therapy reduces the effects of heavy metal poisoning (Kumar et al., 2013). Using L-arginine treatment was able to increase glutathione peroxidase (GPx) activity in mice exposed to Plumbum (Pb) (Tkachenko and Kurhalyuk, 2011). According to Van Harn et al. (2019), reducing crude protein by 1 to 3% of soybean meal with free amino acids (L-Isoleucine, Glycine, and L-Tryptophan) resulted in better growth performance and feed conversion ratio for male broiler chickens. The efficacy of CW in coping against stress was adequate to lower body temperature and increases the circulating blood glucose of chickens during heat spell periods (Abioja and Abiona, 2020).

Although various researchers have studied the ameliorative effect of CW against stress and toxicity using chickens and albino rats, the ameliorative impact of coconut water on toxins against ANF-containing broiler chicken diets has not been tested. Therefore, this study elucidates the ameliorative effects of mature coconut water against ANF-containing *C. merkusii* diet on broiler chickens' body weight, weight gain, feed intake, water intake, survival, and feed efficiency, carcass traits, and organoleptic properties.

Study significance

- Mature coconut water ameliorated stress effect of toxins induced by ANF on *C. merkusii* diet on performance and carcass characteristics of broiler chicken.
- Hence, MCW can serve as water supplement for reducing or preventing the toxic effect of *C. merkusii*-based chicken diets.

MATERIALS AND METHODS

Ethical regulation and study location

The experiment was conducted at the Poultry Laboratory Complex in Mainit Campus of Surigao State College of Technology (SSCT) in Magpayang, Mainit, Surigao del Norte from June 16 to July 21, 2021. The study was supervised by the research committee of the department of agriculture, in compliance with the rules and regulations on the scientific procedures using animals under the Philippines Republic No. 8485, otherwise known as the "Animal Welfare Act of 1998".

Research design, animals, and treatment

Cobb broiler 500 one-day-old (*n*=160) male were purchased from a reliable source and used in this experiment. The chicks were brooded in a communal system. They were fed with maize-soybean-based commercial chick starter crumbles (21% crude protein and 2800 kcal/kg) from days 1 to 7 of age and were managed according to standard broiler production practices and management. On the 8th day, the broiler chicks were randomly assigned to two separate groups. The first group received a standard commercial (maize-soybean) diet with composition (g/kg) was a trade secret by the manufacturer while ingredients and analyzed value were presented in Table 1. The second group of broiler chicks was fed with raw *C. merkusii* root meal (15% giant swamp taro + 85% standard commercial diet) having proximate nutritional composition presented in Table 2.

The experiment was designed by 2 × 4 factorial including two diets (standard commercial vs. ANF *C. merkusii* meal), and four levels of mature coconut water (MCW) as a supplement in drinking water that consisted of 0, 5, 10, and 15%. The chicks were reared in 32-floor pens representing four replicates per treatment. Each pen had five birds with a 0.074 m2/bird floor space allotment. Feeding on experimental diets with levels of MCW in drinking water was initiated from 8 until 28 days of age. The provision of feed and water was ad libitum throughout the experimental period.

Standard diet *and C. merkusii* meal

The standard commercial diets (Sarimanok by UNAHCO, Incorporated, Philippines) were sourced from Agrivet supply stores. The preparation for *C. merkusii* root meal was following the procedures of Taer and Taer (2020) with modifications. Briefly, raw corms were thoroughly washed with tap water, hand peeled, and were finely chopped manually. Thereafter, the chopped corms were subjected to a dehydrator (± 40 ^{0}C) to preserve nutrients (Araújo et al., 2017). Once dried, the chopped corms were ground finely using the hammer mill and the resulting powder was mixed thoroughly with the standard commercial diets. The mixture of *C. merkusii* root meal and standard commercial diets was 15% *C. merkusii* root meal + 85% standard commercial (wt./wt.) basis respectively.

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Table 1 - Ingredients and guaranteed analysis for standard commercial diets based on product label

Starter diet*		Finisher diet*	
Corn		Corn	
Soybean meal		Soybean meal	
Fish meal		Fish meal	
Copra meal		Copra meal	
Corn bran		Corn bran	
Rice middlings		Rice middlings	
Rice bran (D1)		Rice bran (D1)	
Wheat pollard		Wheat pollard	
Cassava meal		Cassava meal	
Sorghum		Sorghum	
Wheat		Wheat	
Salt		Salt	
Calcium carbonate		Calcium carbonate	
Calcium phosphate		Calcium phosphate	
Vegetable oil		Vegetable oil	
Molasses		Molasses	
Dl methionine		Dl methionine	
L-lysine		L-lysine	
Vitamins		Vitamins	
Trace minerals		Trace minerals	
Anti-oxidant		Anti-oxidant	
Mold inhibitor		Mold inhibitor	
Guaranteed Nutrient Analysis			
Crude Protein	Min. 19.50%	Crude Protein	Min. 18.00%
Crude Fat	Min. 5.00%	Crude Fat	Min. 5.00%
Crude Fiber	Max. 4.50%	Crude Fiber	Max. 4.50%
Moisture	Max 12.00%	Moisture	Max 12.00%
Calcium	0.80 – 1.10%	Calcium	0.80 – 1.10%
Phosphorus	Min 0.70%	Phosphorus	Min 0.70%

* Standard commercial (maize-soybean) diet with composition (g/kg) was a trade secret by the manufacturer while ingredients and analyzed value were presented in Table 1.

Table 2 - Proximate nutritional composition of *Cyrtosperma merkusii* root meal

Constituents	*C. merkusii meal*
Moisture	13.91±1.03
Ash	4.82±0.05
Crude Protein	5.94±0.09
Crude fiber	7.15±0.00
Crude fat	0.063±0.005
Nitrogen free extract	68.12±0.020
Metabolizable energy	2635.58±0.070
Calcium	0.50±0.07
Phosphorus	0.073±0.055

Source: Taer and Taer (2020)

Production of drinking water and MCW

The chickens in every group were offered drinking water with 0, 5, 10, and 15% mature coconut water (MCW) concentrations. Water from mature coconuts (*Cocos nucifera* L.) of 10-12 months of age, (Tacunan tall variety) was purchased and used for this study. The preparation of MCW was achieved by modifications of Vani et al. (2021) procedures. Briefly, mature coconuts were dehusked and washed thoroughly with tap water, then air-dried at room temperature (± 25 ^{0}C). Liquid endosperm was collected, filtered the dust and husk particles using the clean and sterile fabric, and stored the filtrate in a refrigerator (± 30 ^{0}F) before using it as a supplement to drinking water for broiler chickens. To avoid spoilage, the unused MCW in the refrigerator was replaced after three days of storage.

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Meat sensory assessment

Samples from breast meat of broiler chickens were obtained for sensory assessments. The meat was steamed (no spices and seasoning) for 30 minutes, and cooked samples were sliced to 10g and served in a clean plastic cup (Taer et al., 2019). Meat sensory attributes viz. tenderness, juiciness, taste, aroma, and general acceptability were evaluated by untrained panel evaluators (n=20) in the Food Laboratory Complex of SSCT, Mainit Campus, Surigao del Norte, Philippines. Using the 5-point hedonic scale (1 being very poor and 5 being very good), the obtained results were tabulated and subjected to ANOVA.

Data collection, measurement and calculation

Live bodyweight of the chickens was measured two times during the 8 D and at the 28 D treatment period. Weight gain was the difference between the starting weight and the ending weight of chickens. Percentage weight gain calculation was done by dividing the weight gain by the initial weight multiplied by 100. Daily feed intake was determined by the weight of feed given minus the refused. Similarly, the water consumption was calculated by the volume of water given minus the leftover. The total feed consumed was divided by the total weight gain of birds to account for the feed conversion ratio (FCR). On termination, two birds per pen were selected, fasted for 10 hours, and then slaughtered. Dress weight was expressed as warm carcass weight (without head, shank, and digesta), while dressing percentage was calculated as a percentage g/kg live weight. The weight of breast and thigh meat was expressed as a percentage of dress weight. The weight of edible organs such as the heart, liver, and gizzard was determined as a percentage of dress weight using the 0.1 g sensitive balance.

Statistical analysis

All data collected having replicate as the experimental unit were subjected to analysis of variance (ANOVA) using International Business Machine (IBM) SPSS Statistics version 26, and the means were separated using Bonferroni Test at $P<0.05$ of the same packages.

RESULTS

Body weight and weight gain

Table 3 shows the effects of MCW supplementation in drinking water of broiler chicken feed standard or ANF diets on live body weight (BW), weight gain (WG), and percent weight gain (%WG). The interaction effect (diet × water) was observed on the final live body weight of broiler chickens. Under the standard and the ANF diets, no differences were noticed among groups regardless of MCW concentrations in drinking water. However, ANF diets tended to have lower live body weights than the standard commercial diet from 8-28 D experimental feeding. The use of top water alone for both standard and feed stress diets likely had a depressed WG (941.25 g and 783.74 g) and lower %WG (69.01% and 67.78%), respectively, compared to MCW supplemented water (Table 3).

Table 3 - Broiler body weight, weight gain, and % weight gain following supplementation of mature coconut water in drinking water under standard or ANF-containing C. *merkusii* root meal diet

Diet	Water	BW (g/bird)	WG (g)	% WG
Standard	Top water	1334.5	941.25	69.01
	5% MCW	1454.25	1051.5	72.34
	10% MCW	1112.25	783.25	69.8
	15% MCW	1411.75	1029.75	72.95
ANF	Top water	1142.25	793.75	67.78
	5% MCW	1323	974	73.44
	10% MCW	1430	993	69.45
	15% MCW	1374.25	1018.5	74.06
SEM		84.136	87.745	3.162
ANOVA	Diet×Water	0.026*	0.916	0.945
	Diet	0.175	0.186	0.32
	Water	0.857	0.227	0.977

*Significant difference (P<0.05), BW= body weight; WG= weight gain; %WG= percent weight gain; MCW= mature coconut water; SEM= Standard error mean; ANOVA= Analysis of variance

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Intake of feed, water, survival rate and FCR

Table 4 presents feed intake, water consumption, survival rate, and FCR affected by supplementation of MCW in broiler drinking water under ANF diets. The results indicated feed intake, water consumption, and survival rate were not significantly different among the ANF diets and/or the MCW water supplements. However, chickens in ANF diets tended to consume more feed and water compared with the standard diet-fed chickens. Significant differences were noticed in the FCR (g feed/g WG) due to supplementation of MCW in drinking water (Table 4). The lowest yet most efficient feed converter was on 5% MCW supplements under standard diet compared to chickens on TW on the ANF diets.

Carcass characteristics

The results for dress weight, dressing percentage, thigh, breast, heart, liver, and gizzards are shown in Table 5. Chicken dress weight was significantly affected due to the interaction of diet x water effects. Chickens supplemented with 10% MCW had the lowest dress weight under the standard diet. However, dress weight for chickens on standard diets tended to be higher over ANF diets. A significant difference for gizzard (% g/g DW) was found between diets. ANF diet groups significantly had higher gizzard as a percentage dress weight over standard diet group. Moreover, chickens on 0 – 15% MCW supplements in the water had a non-significant difference within each diet.

Meat sensory attributes

Table 6 shows the impact of diets, water supplements, and their interactions on customer assessment of broiler chicken breast meat. The main effects of diets, water, and diet*water interactions showed no significant differences as scored by the taste panel on a scale of 1-5. However, meat from chickens given 15% MCW under standard diet was scored as juicier (4.48) than those with counterpart 15% MCW in feed stress (3.93).

Table 4 - Feed, water intake, survival rate and FCR of broiler chicken affected by supplementation of mature coconut water in drinking water under standard or ANF-containing C. *merkusii* root meal diet

Diet	Water	FI (g)	WI (lit)	Survival rate (%)	FCR (g feed/g WG)
Standard	Top water	2085.92	2	95	2.00^{b}
	5% MCW	2077.32	1.74	100	1.73^{b}
	10% MCW	1902.67	2.14	90	2.14^{ab}
	15% MCW	2036.57	1.74	90	1.74^{b}
ANF	Top water	2322.25	2.54	90	2.54^{a}
	5% MCW	2200.28	2.03	100	2.03^{b}
	10% MCW	2180.64	1.87	95	1.86^{b}
	15% MCW	1937.74	1.96	100	1.95^{b}
SEM		121.135	292.721	4.33	0.153
ANOVA	Diet×Water	0.422	0.197	0.364	0.083
	Diet	0.129	0.836	0.422	0.085
	Water	0.302	0.142	0.287	0.043*

$^{a-b}$ Means with varying superscripts differ significantly (P<0.05). * Significant difference (P<0.05). FI, feed intake; WI, water intake; FCR, feed conversion ratio

Table 5 - Carcass characteristics of broiler chickens affected by supplementation of mature coconut water on drinking water under standard commercial or ANF-containing C. *merkusii* root meal diet

Diet	Water	DW (g)	DW (%)	Thigh (%)	Breast (%)	Heart (%)	Liver (%)	Gizzard (%)
Standard	Top water	1087.75	84.38	28.98	30.44	0.67	2.71	1.51
	5% MCW	1082.75	74.44	28.36	30.37	0.73	2.93	1.73
	10% MCW	826.5	76.68	33.86	31.52	0.74	2.88	1.84
	15% MCW	953	67.73	31.16	33.42	0.73	3.04	1.72
Feed stress	Top water	875.25	78.12	32.4	33.56	0.79	3.3	2.38
	5% MCW	1001.75	75.72	28	30.13	0.66	2.68	2.13
	10% MCW	1003	70.13	29.54	32.03	0.7	2.78	2.24
	15% MCW	961.25	69.89	29.69	31.03	0.75	2.96	2.43
SEM		54.574	5.767	2.223	1.647	0.059	0.225	0.165
ANOVA	Diet×Water	0.012*	0.801	0.393	0.43	0.43	0.275	0.407
	Diet	0.488	0.571	0.667	0.831	0.865	0.797	<0.001
	Water	0.156	0.217	0.459	0.63	0.903	0.707	0.79

* Significant difference (P<0.05). DW, dress weight

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Table 6 - Meat sensory attributes of broiler chicken affected by supplementation of mature coconut water in drinking water under standard or ANF-containing C. *merkusii* root meal diet

Diet	Water	Tenderness	Juiciness	Taste	Aroma	General acceptability
Standard	Top water	3.99	3.75	3.86	3.59	3.73
	5% MCW	3.68	3.79	3.9	3.77	3.82
	10% MCW	3.73	3.85	3.65	3.72	3.64
	15% MCW	3.94	4.48	3.96	3.87	3.84
ANF	Top water	3.85	3.82	4.01	4.08	3.95
	5% MCW	3.68	3.64	3.87	3.53	3.62
	10% MCW	3.68	3.78	3.93	3.75	3.81
	15% MCW	3.77	3.93	4.02	3.76	3.77
SEM		0.105	0.182	0.093	0.188	0.102
ANOVA	Diet×Water	0.844	0.384	0.385	0.261	0.158
	Diet	0.222	0.191	0.093	0.752	0.655
	Water	0.099	0.054	0.199	0.76	0.57

DISCUSSION

The study aimed to determine the effects of MCW concentrations in the drinking water of broiler chickens raised under standard or ANF diets. The induction of ANF diets did not influence the body weight, weight gain, and survival of chickens. Similarly, the effects of MCW supplements in drinking water did not affect the same. However, diet*water cross-over interacts significantly with the live body weight of chickens. The bodyweight of chickens was lowest for 10% MCW concentrations under standard diet but turned out to be the highest when offered ANF diets using the same concentration of MCW in water. The BW and WG in ANF diets decreased slightly (1142.25 and 793.75g, respectively) when the chickens received only top water. As the level of anti-nutritional factors ANF in *C. merkusii*-containing diets, broiler chicken performance is more likely to be adversely affected. Anti-nutritional factors can cause detrimental effects on human and animal growth and performance by impairing intake, uptake, or utilization of other foods and feed components or causing discomfort and stress to humans and animals (Bora, 2014). The results of the present study confirmed the many studies using *C. merkusii* and other root meal-based diets in various concentrations (Abdulrashid and Agwunobi, 2009; Getiso et al., 2021; Taer et al., 2022). Anti-nutritional elements commonly observed in all species of the Araceae family are abundant in most parts of the plant, causing throat irritation and mouth epithelium and indirectly reducing the digestibility (Temesgen and Retta, 2015; Taer et al., 2022). However, WG on ANF diets tended to increase (18.50, 20.06, and 22.01%) more than the controls when chickens received 10, 15, and 25% levels of MCW in water (Table 3). Anti-nutritional factors include oxalates, proteinase inhibitors, phytates, tannins, alkaloids, steroids, and cyanogenic glucosides (Kumar, 1992; Steiner et al., 2007; Temesgen and Retta 2015; Hyacinthe et al., 2018; Taer et al., 2022) have a negative impact on digestibility and nutritional quality (Amin et al., 2022) and are low toxic substances that cause severe pathological conditions (Bora, 2014). Having established that the MCW supplemented chickens tended a higher LW and WG performances over the TW chickens given ANF diets is indicative of the protective effects of coconut water (CW) against toxins in the diets. Coconut water has a therapeutic effect (Bhagya et al., 2012; Halim et al., 2018), containing various nutrients such as minerals, vitamins, antioxidants, amino acids, enzymes, and growth hormones (Johnkennedy et al., 2013; Zulaikhah, 2019). Conventionally coconut water has been used as an excellent hydrating drink that maintains the electrolyte balance and helps treat diverse ailments related to oxidative stress, including liver function (Manna et al., 2014). Recently, CW was found rich in L-arginine, a free-form amino acid, and vitamin C, which can prevent heart disease and lipid peroxidation (Bhagya et al., 2012; Prathapan and Rajamohan, 2011). L-arginine can be used for therapy and to reduce the effects of heavy metal poisoning (Kumar et al., 2013). Treatment with L-arginine was able to increase glutathione peroxidase (GPx) activity in mice exposed to Plumbum (Pb) (Tkachenko and Kurhalyuk, 2011). Based on the electrolyte profiling, potassium yielded the highest amount (ranging from 237.41 to 361.20 mg/100 mL), followed by sodium, magnesium, calcium, iron, manganese, copper, selenium, and zinc across varieties of mature coconut water (Halim et al., 2018). Selenium is one of the micronutrients usually acquired through the dietary consumption of plants and animals (Whanger 2002; Eiche et al., 2015) that form the GPx enzyme as a protective agent in neurological and cardiovascular diseases (Lubos et al., 2011). The study of palm oil and coconut water was proven to ameliorate the toxicity-induced cadmium chloride contaminated diet in rats (Mordi et al., 2015) and coconut water against carbon tetrachloride-induced toxicity in rats (Elekwa et al., 2021). According to the Coconut Development Board (CBD), Indonesia, tender coconut water can detoxify toxins in case of poisoning (Zulaikhah, 2019).

In the standard and the ANF diets in each MCW concentration group, treatment-related changes in feed and water intake at the end of experimental treatment were not noticeable. Per survival rate, a slightly decreased in the standard diet group, but this decrease was considered incidental and not a diet-related change. The absence of apparent adverse

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effects of diets agrees with the available literature (Abdulrashid and Agwunobi, 2009) but disagrees with the published findings (Getiso et al., 2021). Water used per kg feed average was 1.74 – 2.14 lit. in standard and 1.86 – 2.54 lit. in ANF diets during the entire trial period. Coconut water stimulates higher water consumption, which ensures the availability of enough water in the body to facilitate evaporative cooling (panting) under hot environmental conditions. Chickens under heat-stress conditions ingested more water (Abioja and Abiona, 2020). This finding was not observed in chickens receiving MCW treatment under ANF diet groups. Broiler chickens on 5% and 10% MCW treatment showed 20.00 and 26.37%, respectively, lower than the controls (Table 4). However, it follows the ratio of water consumption to dry matter intake (DMI), which remained constant at about 2:3 for chickens (Degen et al., 1991; Taer and Taer, 2022).

The effect of MCW supplementation affected the FCR (g feed/g WG) of broiler chickens raised on ANF diets. Chickens in 15% MCW treatment efficiently convert feed into WG ($P < 0.05$) than chickens in TW. The problem with root crop-based diets was lower nutrient densities affecting performance and efficiency (Temesgen and Retta, 2015; Temesgen et al., 2017; Taer et al., 2022), such that the induction of MCW in the drinking water of chickens in this study is speculative to amelioration impact in nutrient-deficient *C. merkusii* diet resulting to improved feed efficiency. Coconut water contains 9 essential amino acids as Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, Threonine, Tryptophan, Valine, and Histidine, while it has 9 Non-essential amino acids such as Alanine, Arginine, Aspartic acid, Cystine, Glutamine, Glycine, Proline, Serine, and Tyrosine (Twishsri et al., 2012). A study of reduced crude protein in 1 to 3% units by replacing soybean meal with free amino acids (L-Isoleucine, Glycine, and L-Tryptophan) resulted in better growth performance and feed conversion ratio for male broiler chickens (Van Harn et al., 2019). Moreover, Coconut water contains growth-promoting phytohormones auxin, cytokinin, gibberellin, inorganic ion, and vitamin (Yong et al., 2009) that play a wide range of developmental processes for plant and mammal general cellular growth (Estevez, 2021). Nitrogen and total protein content of coconut water increased gradually with maturation (Twishsri et al., 2012).

The nutrition of birds has a significant impact on poultry meat quality and safety. In this research, the diet or the MCW as a water supplement did not affect most of the observed carcass characteristics. However, diet × water treatments interact significantly with dress and gizzard weight. Improved gizzards were noted on ANF diet chickens than chickens on standard diets. The result is not consistent with the observation of Abdulrashid and Agwunobi (2009) who reported no significant influence of root meal-based *Colocasia esculenta* diet on gizzard weight. Other studies also reported that dress weight, meat cuts, and other organ weights from root meal-based diets were not significant to those in standard maize-soybean-based diets (Dei et al., 2011; Sultana, 2012; de la Cruz, 2016; Okechukwu and Jiwuba, 2018). Contrarily, some other studies found that root meal-based diets had significant carcass weights, breast, thigh, and edible viscera relative to standard diets (Okpanachi et al., 2014; Getiso et al., 2021; Uguru et al., 2022).

The most influential and perceptible meat features that influence consumers before purchasing meat products are the appearance, texture, juiciness, wateriness, firmness, tenderness, odor, and flavor (Mir et al., 2017). Table 6 showed that 15% of MCW supplementation under the standard diet was juicer among MCW supplements in both diets. Juiciness is related to characteristics of more or less dryness of meat during mastication (Geay et al., 2001). There are two components of juiciness; the sensation of water released during the first bites and juiciness induced by the rapid release of fluid from meat and the influence of lipids on the secretion of saliva (Geay et al., 2001; Chriki et al., 2013). The fatty acid profile of coconut water was capric acid, lauric acid, myristic acid, palmitic acid, stearic acid, oleic acid, and linoleic acid, which is a possible link to juiciness. However, this study does not have enough evidence of the link between CW lipids and juiciness in broiler breast meat. These present findings need substantial evidence in future studies.

CONCLUSION

This study demonstrated the effect of supplementing mature coconut water in the drinking of broiler chickens raised under the ANF *C. merkusii* diets. The influence of MCW treatment on body weight, weight gain, survival, carcass component, and organoleptic traits of broiler chickens under the standard or the ANF diets was insignificant. However, 5-15% MCW treatment within the ANF diets showed an improved FCR better than 0% MCW treatment. The result revealed the ameliorative effects of mature coconut water against anti-nutrients on raw *C. merkusii* root meal inclusion (15% + 85%) in standard diets. Further research is required to corroborate these findings and verify the link between fatty acids and meat juiciness. This will ensure that the nutritional potential of water from mature coconuts can be fully harnessed as a drinking water supplement for broiler chickens.

DECLARATIONS

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Authors' contribution
Cordova S, the sole author who conceptualizes, conducts the investigations, data curation, data analysis and manuscript writing.

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Conflict of Interests

The author has not declared any conflict of interests.

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THE EFFECT OF DIETARY SUPPLEMENTED DRIED FENNEL AND ROSEMARY ON THE PERFORMANCE AND CAECAL MICROFLORA OF GROWING RABBITS

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ABSTRACT: The objective of this study is to evaluate the possible effect of fennel and rosemary dietary supplements on the performance of rabbits. Therefore fifty-six weaned rabbits (40 days old) from white New Zealand breed were divided into two groups and submitted to the following dietary treatments: A) control diet and B) FR diet consisted of 2.5% *Foeniculum vulgare* seeds (fennel) and *Rosmarinus officinalis* leaves (Rosmary) as powder mixed by control diet for thirty days. The growth rate, feed conversion ratio, carcass yield, and mortality were not influenced by dietary fennel and rosemary supplementation. The antimicrobial effect of fennel and rosemary (2.5%) was not observed against *E. coli* in the caecum of the rabbit treated. The essential oils of *Foeniculum vulgare* and *Rosmarinus officinalis* are predominantly composed by Trans Anethole and 1-8 cineole successively. Low antibacterial activity was observed with two essential oils against the strain tested in this study. The addition of the 2.5% combination of fennel and rosemary in rabbit feed did not influence the zootechnical parameters of the rabbits

Keywords: Caecal microflora, Fennel, Growth parameters, Rabbit, Rosemary.

INTRODUCTION

In recent years, some interesting medicinal and aromatic plants in animal nutrition have emerged. The excessive use of antibiotics in feed animals results in bacterial resistance and antibiotic residues in animal products (Oliveira et al., 2020). Phytobiotics represent a part of plants or their bioactive compounds that can be used to improve animal productivity and the quality of food products derived from these animals without danger (Windisch et al., 2008; Alagawany et al., 2021; Ushakova et al., 2021). Their modes of action are still limited. However, antimicrobial activity, antioxidant activity, and the growth performance of phytobiotics were indicated by a lot of studies (Ambar et al., 2018; Łapiński et al., 2018; Aljumaah et al., 2021). Al-Snafi (2018) has reported that *Foeniculum vulgar*e treat abdominal cramps and spastic gastrointestinal disturbances. This aromatic plant has antibacterial, antiviral and antifungal activity (Badgujar et al., 2014). Yan et al. (2010) have showed that the average daily gain (ADG) and feed conversion ratio (FCR) significantly improved during the growing period of pigs using diet supplemented with essential oils rosemary.

Herein, in present study the efficacy of two medicinal plants as feed additives in the diet of rabbit was evaluated.

MATERIAL AND METHODS

Animals and experimental procedure

A total of 56 weaned rabbits white New Zealand (40 days old; male and female; 900 ± 100 g initial body weight), were divided into two groups and submitted to the following dietary treatments (Table 1) for twenty-one days. The first group was control diet, the second group was FR (2.5% *Foeniculum vulgare* and *Rosmarinus officinalis* powder mixed with the control diet). The two groups take only the control food for the remaining ten days.

The rabbits were kept in standard cages with 2 animals per cage (2010/63/EU Official Journal of the EU 2010) in a building with temperatures between 15 and 20°C, and humidity levels between 60% and 70%. The duration of daily illumination was 16 h. The rabbits had access to feed and water ad libitum. The bodyweight of rabbit's feeds consumption and mortality rates were measured every week during the experiment.

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Table 1 - Ingredients and chemical composition and nutritive value of basal diets

Ingredients	Control diet (%)	Fennel and Rosemary diet (%)	Chemical composition (g/100 g)	Control diet	Fennel and Rosemary diet
Bran	29	28.275	Dry matter	89.92	89.87
Corn	10	9.75	Ash	8.03	8.06
Soybean meal	4	3.9	Crude protein	16.77	16.7
Sunflower meal	5	4.875	Ether extract	2.66	2.82
Alfalfa	40.5	39.487	Crude fiber	14.5	14.7
Barley	9	8.775	Neutral detergent fibre	31.7	32
Salt	0.5	0.48	Acid detergent fibre	18.1	18.5
Premix *	1	0.975	detergent lignin	4.77	5.01
DL Methionine	0.05	0.048	Digestible energy (Kcal/Kg)	2270	2274
L-Lysine	0.25	0.24			
Dicalcium phosphate	0.61	0.59			
Calcium carbonate	0.09	0.08			
Fennel	0	1.25			
Rosemary	0	1.25			

*One kilogram of Premix provides: 1000000 IU vit.A, 300000 IU vit. D, 2 g vit. E, 0.4 g vit. K, 0.075 g vit. B1, 247 0.4 g vit. B2, 1.218 g vit. B3, 0.099 g vit. B5, 0.083 g vit. B6 , 0.190 g vit. B9, 0.030 g vit. B12, 0.005 g Biotin, 248 0,2 g Cuivre, 4 g Fer, 5 g Zinc, 0.012 g Iode, 0.012 g Selenium, 0.020g Cobalt, 6 g Manganese, 57 g Choline 249 chloride and QSP calcium. Premix contained 50 ppm of Salinomycin.

Chemical analysis

Chemical analysis of diets was calculated with Spanish foundation for the development of animal nutrition (Fundación Española para el Desarrollo de la Nutrición Animal, FEDNA) table of composition and nutritive value of ailments (Blas et al., 2010). The essential oils (EO) of each plant were extracted via steam distillation for 3 hours using a Clevenger-type apparatus. Chemical analysis of essential oils was done by gas chromatography coupled with mass spectrometry.

Bacteriological analysis

Bacterial counts

Three animals from each group were slaughtered on 40, 50, and 60 days. Bacteria from caecal samples were isolated by the standard microbiological method using the appropriate dilutions in the Ringer solution. Dilutions were plated onto the Mac Conkey agar for *E. coli*, incubated at 37°C for 24 hours. The bacterial counts were expressed in colony-forming units per gram (log 10 CFU · g–1).

Isolation of E. coli bacteria

Specimens collected from the carcasses of rabbits (not submitted to previous dietary treatment) showed prior signs of diarrhea and bloating. Miniature biochemical tests can be conveniently and simultaneously performed on a colony for *E. coli* identification using an API 20 e gallery.

Antibacterial activity

A preliminary assay was performed with the agar diffusion method to compare the antibacterial effects of the essential oils against the performance of the antibiotic Oxytetracycline. The diameters of the resulting inhibition zones were measured in centimeters, including the diameter of the well. The minimum inhibitory concentration (MIC) was defined as the low essential oil concentration capable of producing a total inhibition of growth after an incubation period of 24 to 48 hours (Remmal et al., 1993). The minimum bactericidal concentration (MBC) was defined as the minimum bactericidal concentration of the oil capable of killing the inoculum. The MIC1 and MBC2 values were determined by micro broth dilution assay using resazurin as 80 an indicator (Mann and Markham, 1998) of bacterial growth.

Statistical analysis

The results were given as mean ± standard deviation (SD), statistical evaluation of the results was performed by one-way ANOVA along with the level of significance set at $P < 0.05$ and the Chi-Square test for mortality.

RESULTS

The body weight, growth rate, feed intake, feed conversion rate, carcass yield and mortality of rabbits during the experiment are presented in table 2. The digestible energies of Control diet C and feed FR are iso-protein, and iso-energetic are very low compared to those recommended by Lebas (2004).

The weight of animals fed FR tended to be somehow higher than that of animals fed Control diet. The FR group consumed a little more than the C group, especially after the 2nd week of treatment. There was no significant difference

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in the growth rate of the animals given the FR diet or the animals given the Control diet. The feed conversion rate is similar in the two groups during the 21 days of treatment. The carcass yield and livers of rabbits did not change significantly with the addition of the 2.5% combination of fennel and rosemary in their feed. Moreover, the digestive disorders that characterize the weaning period manifested by severe diarrhea and put animal health in danger. Mortality in the FR group was better than that recorded in the control group C.

Effects of dietary fennel and/or thyme on caecal microbial counts are presented in Table 3. There was no significant difference in the number of *E.coli* in the cecum of rabbits fed control diet or FR diet. The chemical composition of the essential oils of *Foeniculum vulgare* and *Rosmarinus officinalis* are shown in Table 4. The essential oil of *Foeniculum vulgare* seeds is dominated by the trans anethole compound and by fenchone. The fundamental component of the essential oil of *Rosmarinus officinalis* is 1-8 cineol, together with α-pinene, the α-phellandrene, and camphene.

The results of the antibacterial activity of essential oils against *E. coli* are shown in Table 5. The essential oil of the rosemary tested did not show an *E.coli* antibacterial effect. The combination of the essential oil also did not demonstrate antibacterial power against these bacteria. This gives reason for the low impact of these phytobiotics on *E.coli* bacteria in the cecum. The addition of the 2.5% combination of fennel and rosemary in rabbit feed did not influence the zootechnical parameters of the rabbits.

Oxytetracycline and *Rosmarinus officinalis* essential oil did not have activity against *E. coli*. The essential oil of seeds of the *Foeniculum vulgare* showed low activity against the bacteria with 1.4 cm zone of inhibition. The combination of the two essential oils *Foeniculum vulgare* and *Rosmarinus officinalis* was to be ineffective against *E. coli*.

The minimum inhibitory concentrations (MIC) and bactericidal (MBC) of essential oils against *E. coli* are grouped in table 6. The essential oil of *Foeniculum vulgare* showed the lowest antibacterial effect (MIC = 50% v/v) against *E.coli* with no bactericidal action at the concentrations tested. The essential oil of *Rosmarinus officinalis* was inactive concerning the strain tested in this study (MIC > 50 M L/ml).

Table 2 - Effect of dietary supplementation with fennel seeds and rosemary leaves on rabbit growth performance and mortality

Traits \ Group (mean ± SD)	Days	Control diet	Fennel and Rosemary diet	P value
Body weight (BW), g	40	880±80	916±130	0.217
	47	1143±100	1164±170	0.598
	54	1321±165	1414±200	0.162
	61	1501±190	1619±209	0.152
	71	1821±159	1943±207	0.191
Feed intake, g. d–1	40	81.4±7	76.5±6	0.059
	47	97.5±12	99±7	0.666
	54	101.7±11	108.4±19	0.271
	61	134±9	138±10	0.647
	71	134.2±14	137.7±13	0.649
Growth rate, g. d–1	40-47	39.1±13	37±13	0.560
	47-54	42.9±32	35.2±9	0.399
	54-61	35.6±10	38.6±6	0.402
	61-71	37.2±10	33.9±6	0.413
Feed conversion ratio	40-47	2.36±0.7	2.49±0.9	0.588
	47-54	3.14±0.9	3.22±0.6	0.797
	54-61	3.36±0.9	3.33±0.5	0.082
	61-71	3.74±0.8	4.1±0.7	0.409
Carcass yield, (% of BW)	50	56.4±3	51.4±6	0.294
	60	55.6±5	52.9±3	0.445
	71	54.4±1	52.9±1	0.114
Liver yield, (% of BW)	50	3.7±0.4	3.8±0.3	0.672
	60	3.6±1	4.1±1	0.584
	71	4 ±0.1	4±0.2	0.605
Mortality [1] ,%	40-71	42.9	46.4	> 0.05

[1] mortality are analysed using a χ 2 test at P < 0.05

Table 3 - Counts of *E. coli* in caecum of rabbits (log 10 cfu . g–1; mean ± SD)

Days	Bacteria	Group		P value
		Control diet	Fennel and Rosemary diet	
40	*E.coli*	3.3 ±0.3		
50	*E.coli*	3.94±0.2	4.35±0.3	> 0.05
60	*E.coli*	3.93±0.3	3.79±0.3	> 0.05

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Table 4 - Chemical composition of essential oil			
Component	**Retention time (min)**	***Foeniculum vulgare* seeds**	***Rosmarinus officinalis***
Myrcene	6.55		
Para cymene	7.19		
cis-Ocimene	7.73	0.41	
α-Pinene	7.75		
γ terpinne	7.79		
α-Pinene	8.28		18.94
Linalol	8.51		
Camphene	8.74		5.38
α-Phellandrene	9.70		5.46
Terpinène -4-ol	9.79		
α-Pinene	11.01	2.22	
dl-Limonene	11.01		
Thymol	11.38		
Carvacrol	11.55		
1-8 cineol	11.68		51.62
Fenchone	12.99	6.14	
Fenchone	13.00		
β-Caryophyllene	13.08		
α–Campholene aldehyde	15.63		10.65
Borneol	16.47		2.59
Isopulegyl acetate	17.81		
α-Fenchyl acetate	18.23		
Trans Anethole	20.07	91.12	
Trans Anethole	20.10		

Table 5 - Diameter of inhibition zones of essential oil against *E.coli* (cm)				
Bacterial strain	**Antibiotic**	**Essential oil**		
	Oxytetracycline	***Foeniculum vulgare***	***Rosmarinus officinalis***	***Foeniculum vulgare* +*Rosmarinus officinali***
E.coli	0.6±0	1.4±0.6	0.6±0	0.9±0.5

Table 6 - Minimum inhibitory concentration, minimum bactericidal concentration en µl/ml.				
Bacterial strain		**Essential oil**		
		Foeniculum vulgare	***Rosmarinus officinalis***	***Foeniculum vulgare* + *Rosmarinus officinalis***
E.coli	CMI	50	>50	>50
	CMB	>50	>50	>50

DISCUSSION

The efficacy of combination of fennel and rosemary in rabbit feed was not showed in this work. Several studies have demonstrated positive effects of phytobiotics as feed additives in the diet of animals. Omer et al. (2013) found that adding 0.5% fennel seed with 0.5% oregano leaves as feed additives improved performance parameters of rabbits. Ghazalah and Ali (2008) results confirm the significant improvement in weight as well as in daily weight gain in chicks fed a feed supplemented with 0.5% leaves of *Rosmarinus officinalis*. They noticed that with this dose the zootechnical parameters are better than higher doses (1.2%). Likewise, the study by Al kassi, (2008) showed this positive effect of daily weight gain but with the use of 1% of *Rosmarinus officinalis* in chicken feed. Abdullah et al. (2009) also observed that supplementation of broiler diets with 1, 2 and 3 g/Kg of fennel seeds improved weight gain. Cesari et al. (2008) found a significant improvement in rabbit weight, daily weight gain, and conversion index and the use of the combination of organic acids and essential oils from rosemary, thyme, and cinnamon. Mathlouthi et al. (2012) noticed these positive effects with the addition of 50 mg/Kg in chicken feed. However, Erdelyi et al. (2008) did not find a positive or a significant impact on these zootechnical parameters of rabbits within the use of essential oils from rosemary and garlic. The mortality performance results were close to the results obtained by Erdelyi et al. (2008) as 48% that incorporated 0.15% of the essential oil of *Rosmarinus officinalis* in the diet of rabbits. However, Benlemlih et al. (2020) demonstrated that fennel dietary supplements (5%) on the feeding of rabbits decreases mortality. According to Cesari et al. (2008)'s study, the mortality showed to be reduced to 42% compared to the control group within the use of a combination of organic

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acids and rosemary's essential oil, thyme, and cinnamon. Fotea et al. (2009) noticed that the mortality of chickens decreased with the 0.5% dose, better than with 1 and 1.5% rosemary added to their feed. The lower dosage used in this experiment with the low antibacterial activity of the combination of the essential oil of *Rosmarinus officinalis* and *Foeniculum vulgare*, did not prevent the breeding of the high mortality rate. Moreover, the essential oil combination also did not demonstrate antibacterial power against *E.coli*. which gives reason for the low impact of these phytobiotics on this bacterium in the cecum. However, reduced counts of *E. coli* was showed by Benlemlih et al. (2014) during the application of dried fennel and thyme to rabbit.

Following our results, the essential oil yield of the *Foeniculum vulgare* (2.11 %) and *Rosmarinus officinalis* (0,49%) was similar to values published by Stefanini et al. (2006) for the Brazilian fennel (2.7%) and by Moujahed et al. (2011) for the Tunisian rosemary (0.43%). This study showed that the essential oils of *Foeniculum vulgare* are distinguished by a large percentage of the phenol phenylpropanoids. The essential oil of *Rosmarinus officinalis* consists of terpenes and oxygen-containing terpenes. The low antibacterial activity recorded by essential oils of seeds and leaves of *Foeniculum vulgare* has already been demonstrated by the study of Grigore et al. (2012). The essential oil of *Rosmarinus officinalis* was ineffective against our isolated bacterium despite its capacity in oxygenated monoterpenes (51%). Ait-Ouazzou et al. (2012) studied the antimicrobial effect of 11 major constituents of essential oils and found that the 1.8 cineole had moderated activity compared with other oxygenated monoterpèniques compounds such as carvacrol, thymol, and, linalool.

The yield of rosemary essential oil is very low. In our phytobiotic the small content of essential oil contained only (0.038%) within the addition to its low antibacterial activity which may explain its negativity on mortality and its power on growth and other parameters.

CONCLUSION

The addition of the 2.5% combination of fennel and rosemary in rabbit feed did not influence the zootechnical parameters of the rabbits. The presence of Trans anethole in fennel essential oil involves in reduction of the number of *E. coli* suggested that other doses of fennel as feed additives should be studied.

DECLARATIONS

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Authors' contribution
All authors contributed to research conduction, analyzing and writing equally.

Conflict of interests
The authors declare that there is no conflict of interests in this work.

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MORPHOLOGICAL AND MORPHOMETRIC FEATURES OF INDIGENOUS CHICKEN IN SOUTHWEST ETHIOPIA

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ABSTRACT: Morphological and morphometric characterization of indigenous chicken ecotypes were carried out in West-Omo zone of Southwest Ethiopia. Nine qualitative and fifteen quantitative traits were observed and measured from 660 matured chickens of both sexes. The data was analysed using SPSS version 21 and SAS version 9.1. Majority of the qualitative and quantitative traits were significantly influenced by sex and agro-ecological zones. The predominant plumage color, feather distribution, shank color, skin color, ear-lobe color, eye color, head shape, comb type, and feather morphology were red (38.4%), normal (96.2%), yellow (45%), white (48.8%), red (42.1%), red (28.6%), flat plain (94.4%), single (64.7%) and normal (100%). The body weight of matured male chickens in highland, mid-altitude and lowland agro-ecologies was 2.1±0.02 kg, 2.2±0.05 kg, and 2.0±0.03 kg, respectively, while females weighed 1.4±0.01 kg, 1.5±0.00 kg, and 1.4±0.01 kg in highland, mid-altitude, and lowland agro-ecologies, respectively. Males were also superior to females in terms of body length (BL) values of 42.0±0.19 cm and 37.7±0.06 cm, respectively. The prediction of body weight could be based on regression equation y = -1.02+0.10 CC (chest circumference) for male and y= -1.26+0.07BL of hen in highland altitude, y = -1.06+0.11 CC of male and y= -0.78+0.05 BL of hen in mid-altitude and similarly y = -0.90+0.10 CC in lowland male and y= -1.33+0.07 BL of lowland hen. Therefore, chest circumference for males and body length for females were the best variables to predict the body weight of chickens than other variables. The current finding shows there was heterogeneity in a population of indigenous chickens in the studied agro-ecology. This gives an opportunity for genetic improvement of indigenous chickens within a population.

Keywords: Genetic improvement; Indigenous chicken; Morphological; Morphometric; West-Omo zone.

INTRODUCTION

Ethiopia is a home for at least seven indigenous chicken ecotypes namely Farta, Horro, Jarso, Konso, Mandura, Tepi, and Tillili (EBI, 2016), with an estimated 60.04 million poultry heads. Despite the low productivity, the indigenous chicken population represents 88.5% of the poultry flock in Ethiopia (CSA, 2018). Indigenous chickens are resistant to common poultry diseases and feed quality and quantity fluctuation, requiring minimal input (Desta and Wakeyo, 2012; Desta, 2021). More than 90% of the country's egg and meat production is produced by indigenous chicken managed in the traditional way (Melesse and Negesse, 2011). However, only a few recognized chicken breeds have a fair description of their physical appearance, as well as indications of their level of performance, reproduction, and genetic characteristics. Thus, a basic understanding of a livestock species' or breed's defining characteristics that distinguish out from other breeds or species is required for genetic improvement and designing an appropriate breeding plan (Oguntunji and Ayorinde, 2015; Bibi et al., 2021).

Characterization of farm animal genetic resources is a strategy for identifying several breeds or populations in a particular production zone by defining their morphological and productive characteristics (FAO, 2012). It has also been revealed that, distinct breeds will be expected to boost the number of livestock breeds in the country (Georges et al., 2019). Morphometric measurements have been applied to identify the types of different livestock breeds and could generate preliminary evidence for the choice of a particular breed (Mwacharo et al., 2006). On-farm characterization help to ensure the long-term improvement and conservation of indigenous animal genetic resources, and it's becoming more popular in determining variation between and among the breeds (Alderson, 2018; Dobrzański et al., 2019).

There have been morphological and genetic characterization works on indigenous chicken ecotype found in Sheka zone of Southern nation and nationality people regional state of Ethiopia (Assefa and Melesse, 2018a). However, there is a scarcity of such information documented by morphological and morphometric evaluation across the various agro-ecologies of the study sites. Thus, it is believed that in such remote areas, genetic originality may still be found. The phenotypic features of distinct breeds are thus critical as a foundation for establishing long-term genetic improvement approaches. Therefore, this study aimed to systematically identify the morphological and Morphometrical characteristics of indigenous chickens reared under the different agro-ecologies of West-Omo zone of southwest Ethiopia.

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MATERIALS AND METHODS

Animal care and ethical issues

Mizan-Tepi University, College of Agriculture and Natural resource ethics Committee approved the experiment (1956ET-18/2021) after a careful assessment of ethical and animal care issues. Directive 2010/63/EU of the European Union guidelines (2010) concerning the treatment and use of animals for research and development purposes were employed.

Description of the study areas

The research was carried out in Ethiopia's Maji and Bero district, West-Omo zone (WOZ) of the South Nation's Nationalities and Peoples Regional State (SNNPRS). The districts were chosen based on their chicken population potential as well as their production environment. The detailed description is fully explained as follows (Table 1).

Table 1 - Description of study areas

Description	Bero District	Maji District
Geographical Location	06˚ 15.213ˈN and 35˚13.449′ E	6°12′N and 35°35′E
Temperature (ºC)	20.1- 27.5 C˚	15.1- 27.5 C˚
Annual rainfall (mm)	1,401 to 1,800	400-1800
Altitude (m.a.s.l)	501 to 1,750	500–2500
Chicken population (head)	174,075	226,772

Source: WOZADMD, 2019.

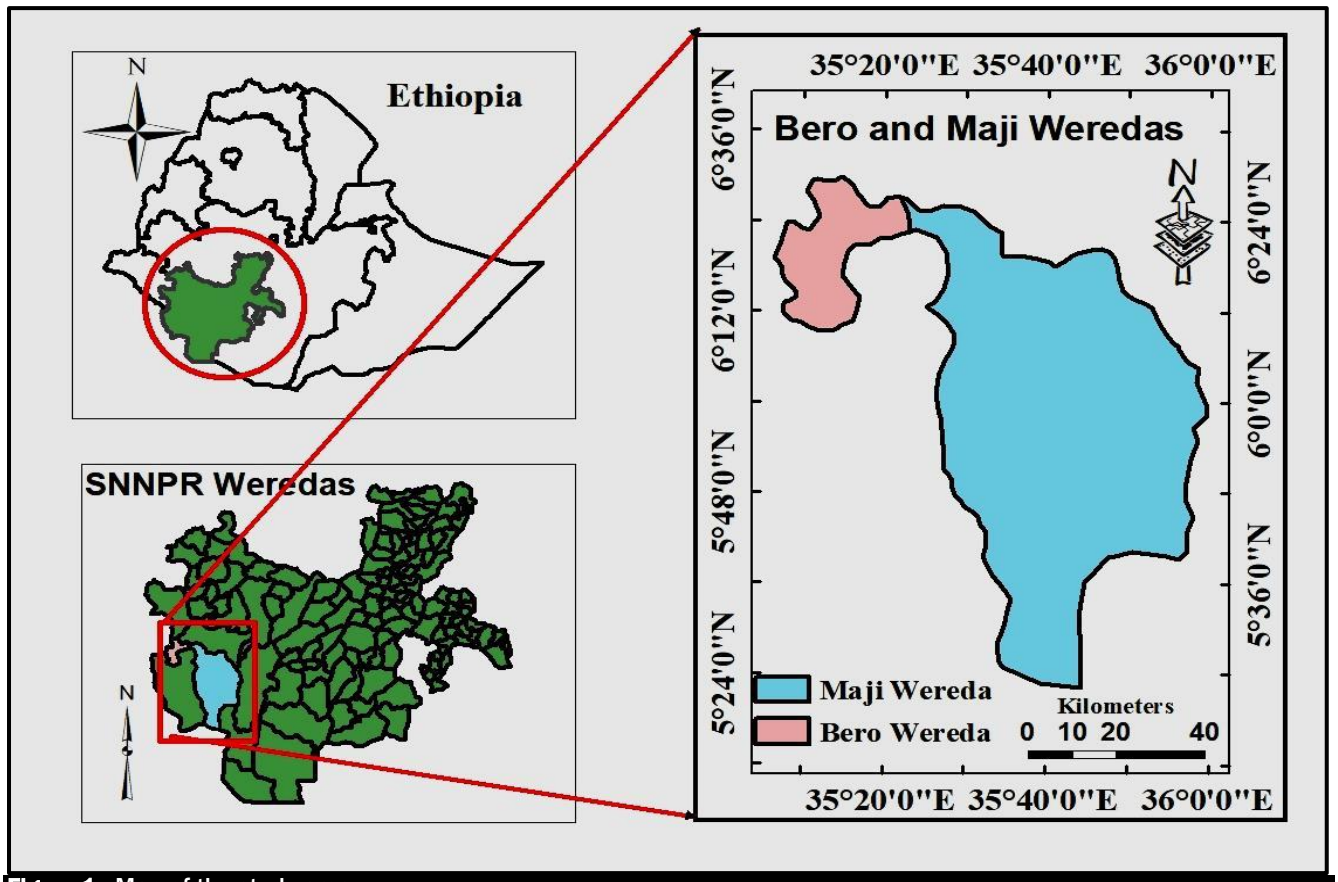

Figure 1 - Map of the study area

Site selection and sampling techniques

Prior to sampling, key informants and zonal livestock and fisheries resource experts were consulted to understand more about the genetic diversity of indigenous chicken in the study area. A quick field survey was conducted to determine the distribution of indigenous chicken breeds in the study area and to design a sampling framework from which sampling units were taken. A multistage purposive sampling technique was used to identify samples in the study zone. West-Omo zone is structured into seven districts and one urban town which was stratified and purposely selected based on its chicken population. Of these Bero and Maji districts were purposively selected and stratified into three agro-ecological

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zones of highland, mid-altitude, and lowland, to assess the effect of agro-ecologies on the morphology of chicken ecotype. Then a total of six kebeles were selected purposively from both districts (two kebeles from each agro-ecologies) based on suitability for chicken production, accessibility to market and road, security, and willingness of the farmers to participate in the study. Thus, a total of 660 chickens were considered for both qualitative and quantitative trait studies. Animals samples were identified in accordance with FAO (2012) guidelines and the morphometric investigation comprised chickens belonging to 240 households.

Data collection

Quantitative and qualitative traits

Data on qualitative (morphological features) and quantitative (morphometric measures) variables were gathered and documented using a format based on the FAO's standard description list (FAO, 2012). As per the visual observation, a total of 9 qualitative characteristics were measured and recorded (Figures 2-5), including Plumage Color (PC), Shank Color (SC), Feather Morphology (FM), Feather Distribution (FD), Skin Color (SkC), Earlobe Color (ELC), Eye Color (EC), Head Shape (HS) and Comb Type (CT). Likewise, 15 quantitative traits/parameters were measured and recorded using measuring tape and a measuring stick, which included Body weight (BW), Body Length (BL), Chest Circumference (CC), Wing Span (WS), Neck Length (NL), Shank Length (SL), Shank Circumference (SC), Thigh Circumference (TC), Wattle Length (WL), Wattle Width (WW), Comb Length (CL), Comb Height (CH), Beak Length (BkL), Beak Width (BkW) and Height at Back (HB) were taken measurements at early in the morning to avoid the effect of feeding and watering on the chickens weight. Less than or equal to 4 chickens per household/farmer were chosen to avoid genetic resemblance. Two researchers carried out the measurements, one taking the measurements and the other collecting data. All measures were obtained by the same researcher throughout the investigation to reduce subjective error.

Data analysis

The frequency technique PROC FREQ in the Statistical Package for the Social Sciences (SPSS, ver. 21) was used to examine different measurements of qualitative morphological features. The Chi-square (x2) test was performed to determine if there was a significant relationship between the categorical variables. Quantitative data (body weight and linear body measurement) were subjected to GLM (Generalized Linear Model) procedures of Statistical Analysis System (SAS, 2008 ver. 9.1) by fitting agro-ecology and sex as independent variables. For each physical attribute across agro-ecology and sex, the least square means and standard errors were determined.

Body weight and linear body measurements of female and male individual chickens were determined using the following model:

$$Yijk = \mu + Ai + Sj + (AxS)ij + eijk$$

Where:

Yijk = the observed k (body weight or linear body measurements) in the ith agro-ecology and jth sex

μ = overall mean

Ai = fixed effect of ith agro-ecology (Highland, Mid-altitude, Lowland)

Sj = fixed effect of jth sex (male, female)

(AxS)ij= the interaction effects of ith agro-ecology and jth sex

eijk = random error

The following models were used for the estimation of body weight from linear body measurements.

For males:

$Yj = \beta0 + \beta1\ X1 + \beta2\ X2 + \beta3\ X3 + \beta4\ X4 + ej$ for males

Where: Y = the dependent variable (body weight); β0= the intercept;

X1, X2, X3 and X4 are the explanatory variables (chest circumference, thigh circumference, body length and shank length)

β0= the intercept

β1 ..., β4 are regression coefficients of the variables X1..., X4

ej = random error

For females:

$Yj = \beta0 + \beta1X1 + \beta2X2 + \beta3X3 + \beta4X4 + \beta5X5 + \beta6X6 + eij$ for females

Where: Yj = the dependent variable (body weight);

β0= the intercept

X1, X2, X3, X4, X5, and X6 are independent variables (Body length, neck length, shank circumference, height at back, wattle length, and thigh circumference);

β1... β6 are regression coefficients of the variables X1..., X6

ej = random error

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RESULT AND DISCUSSION

Qualitative traits in the study zone

The physical qualities of livestock breeds must be described in detail to form a breed and design a breeding plan for a certain production system (Machete et al., 2021; Tadele et al., 2019). Morphological features of the indigenous chicken population in the study agro-ecologies are summarized in Table 2. The most predominant plumage color, feather distribution, shank color, skin color, ear-lobe color, eye color, head shape, comb type, and feather morphology were red (38.4%), normal (96.2%), yellow (45%), white (48.8%), red (42.1%), red (28.6%), flat plain (94.4%), single (64.7%) and normal (100%). According to the Chi-square test, the frequency distributions among the three agro-ecologies were significantly different ($P<0.05$) with respect to all qualitative traits except for skin color.

Plumage color variation

Male and female indigenous chickens with diverse plumage colorations are shown in Figure 2. Brown plumage color was predominant among female chickens in the mid-altitude zone (39%), whereas red plumage color was observed in both highland and lowland agro-ecologies with 42% and 39%, respectively. For male chickens, the predominant plumage color was red mixed with black (45%) in mid-altitude, red (95%) in highland, and red (35%) in lowland agro-ecologies. The variation in plumage color might be due to a farmer's traditional selection method, the environment, or genetic variation. Similar to the current finding, Assefa and Melesse (2018b) find that male chickens in Yeki district have red plumage color (37.5%). Tadele et al. (2019) also found that red was the major plumage color of male chickens throughout the study districts of Kaffa zone, accounting for 59.3%, while reddish-brown plumage color was prominent for females in all districts. However, our findings are contradicted with the value of Alebachew et al. (2019) in the Benshangul Gumuz area of western Ethiopia, who found that white (39%) was the most common plumage color for Bambassi ecotypes, followed by black (12.7%) and gray (12.7%). The current finding also contradicts with Getachew and Negassi (2016) who found that roughly 58.3% of male chicken populations in the north-bench district had black plumage, followed by white, Gebsima (15.0%), and red plumage (11.9%).

Eye color variation

Orange eye color (Figure 2) was predominant for male and female chickens in all agro-ecologies with an overall proportion of 41.7% followed by red (28.6%) yellow (26.8%), brown (1.1%), and whitish pale (1.8%). Similarly, Aklilu et al. (2013) found that orange eye color (wild-type color) was observed in higher frequency in Horro chicken (87.84%), followed by the red eye color. The pigmentation (carotenoid pigment) and blood flow to a variety of structures within the eye play significant roles in eye color variation (Crawford, 1990).

Skin color variation

Four skin colors (Figure 3) namely white, yellow, black, and grey were observed (Table 2) of which 57.3 % of chickens in highland areas had skin with white color followed by 47.2% and 41.8% in mid-altitude and lowland agro-ecologies, respectively. Likewise, Aklilu et al. (2013) indicated that most of the local chickens observed in Horro district had white (77.03%) skin color followed by yellow (22.07%) and bluish-black (0.9%). Rajkumar et al. (2017) also found that skin color variations of white, pink, and yellow were observed in indigenous chicken populations, and that white skin color was the most prominent among them. The finding disagrees with Churchil et al. (2019) who reported that the skin color was 100% yellow for Aseel male chicken in India. According to Eriksson et al. (2008), the presence or absence of carotenoid pigments results in yellow or white skin. Domestic hens with yellow skin are homozygous for a recessive gene that inhibits the synthesis of an enzyme called BCDO2 (beta-carotene dioxygenase 2) in comparison to white chickens with the dominant allele. This recessive gene may have been introduced from Grey Jungle fowl (*Gallus sonnerati*).

Head and feather morphology

There is a significant ($P<0.01$) relationship between chicken feather morphology and agro-ecologies (Figure 4). The feather morphology of local hens, regardless of sex, was found to be 100 % normal among the agro-ecologies. The proportion of flat head chicken was dominant in all agro-ecologies with an overall percentage of 94.4% while, the remaining proportion (5.6%) accounted for Gutye (crested) head shape. The findings were similar to that of Nigussie et al. (2015), who found that the predominant head shape of local chickens across agro-ecology was flat heads, with 98.2%, 92.7% and 92.3%, respectively from highland, mid-altitude, and low land, and the rest was Gutye (crested) head-shaped. In Makurdi, Egahi et al. (2010) obtained 82.05 percent for the plain head shape type of native chickens. Kibret (2008) on the other hand, founds 48.82% and 51.18% for plain and crested head shape types, respectively

Comb type variation

Four comb types, single, double, pea, and rose were identified in the order 64.7%, 10.3%, 5.6%, and 19.4%, respectively (Table 2; Figure 4). The current study revealed that the single comb type was predominant accounting for 36.3 %, 76.8%, and 80.9% in highland, mid-altitude, and lowland agro-ecologies, respectively followed by the rose comb type, accounting for 35.4 %, 12.3%, and 10.5 % in the agro-ecologies mentioned. Consistent with the current finding, Assefa and Melesse (2018) reported that single comb types for mid-latitude and lowland were found in Sheka indigenous chicken. Moreover, Emebet et al. (2014) indicated that single and rose comb types were present in 59.2% and 31.8% of chickens in the Southwest and South regions of Ethiopia, respectively. In contrast to our finding, Churchil et al. (2019) pea comb type is dominant for Aseel chicken in India. Comb size is linked to gonadal development and light intensity, although comb type is a result of gene interaction (Bell and WeaverJr., 2002).

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Shank color variation

According to the current study, four shank colors were identified namely yellow, grey, white, and black shank color (Figure 5). Yellow color occurred highest (45 %) followed by grey color (38.6%), white color (12.9%), and black color (3.5%). Similarly, Mogesse (2007a) reported yellow shank color as the highest in Ethiopian native chickens. Mancha (2004), on the other hand, found that the most prevalent shank colors in Plateau State were pink, dark-ash, ash, and pale yellow.

Ear lobe color variation

Earlobe color had a significant (P<0.01) relationship across all the study agro-ecologies (Figures 2-4). Five types of earlobe colors (red, white, yellow, grey, and dark brown were identified among the indigenous chickens with 42.1%, 32.7%, 21.7%, 2.3%, and 1.2%, respectively) were identified among the indigenous chicken in the study area. Male ear lobe color was yellow (50%) in the lowland, whereas red was more common in the highland and mid-latitude regions, with values of 50% and 50%, respectively. Female ear lobe color was red (as 67%) in the highland, but white (as 48% and 41%) in the mid-altitude and lowland, respectively. The finding of the current study was comparable with reports on Assel chicken (Rajkumar et al., 2017) and indigenous Shaka chicken (Assefa and Melesse, 2018).

Black plumage with black shank colour

White plumage with orange eye colour

Brown plumage colour

The dominant plumages colour

Figure 2 - Pictures showing plumage color of indigenous chickens in West-omo Zone

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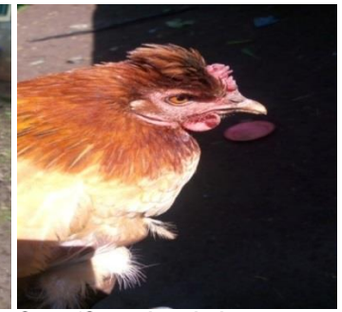
Yellow skin necked neck

Black skin naked neck

Red skin naked neck

Figure 3 - Pictures showing skin colors of indigenous chickens in West-omo Zone.

Single comb type



Crest/Gutye headed type

Rose comb type

Figure 4 - Pictures showing comb structure and head type of indigenous chickens in West-Omo zone

Black shank colour

Yellow shank colour

Grey shank colour

White shank colour

Figure 5 - Picture showing shank color of indigenous chickens in West-omo Zone.

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Table 2 - Qualitative characteristics of chickens in the study agro-ecology

Traits	Attribute	Agro-ecology																		Overall	
		Highland						Mid-altitude						Lowland							
		F		M		T		F		M		T		F		M		T			
		N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
Plumage color	Red (*Key*)	84	42	19	95	103	46.8	62	31	4	20	66	30	78	39	7	35	85	38.6	254	38.4
	Golden	12	6	-	-	12	5.45	23	11.5	3	15	26	11.8	20	10	1	5	21	9.5	59	8.9
	Gebsima	8	4	1	5	9	4	3	1.5	4	20	7	3.18	3	1.5	3	15	6	2.7	22	3.3
	Brown	64	32	-	-	64	29	78	39	-	-	78	35.45	61	30.5	1	5	62	28.1	204	34
	Black (*Tikur*)	22	11	-	-	22	10	6	3	-	-	6	2.7	10	5	1	5	11	5	39	5.9
	Grey	6	3	-	-	6	2.7	2	1	-	-	2	0.9	7	3.5	1	5	8	3.6	16	2.4
	Tikur teterma	-	-	-	-	-	-	3	1.5	-	-	3	1.36	2	1	-	-	2	0.9	5	0.75
	White	-	-	-	-	-	-	4	2	-	-	4	1.8	-	-	-	-	-	-	4	0.6
	Kokima	4	2	-	-	4	1.8	13	6.5	-	-	13	5.9	4	2	-	-	4	1.8	21	3.2
	Red with black	-	-	-	-	-	-	2	1	9	45	11	5	9	4.5	6	30	15	6.8	26	3.9
	Multicolor	-	-	-	-	-	-	4	2	-	-	4	1.8	6	3	-	-	6	2.7	10	1.5
Test	X^2 and P-value																			67.9	***
Eye color	Red	68	34	4	20	72	32.7	74	37	4	20	78	35.4	36	18	3	15	39	17.7	189	28.6
	Yellow	48	24	4	20	52	23.6	34	17	6	30	40	12.18	78	39	7	35	85	38.6	177	26.8
	Orange	76	38	12	60	88	40	88	44	9	45	97	44	80	40	10	50	90	40.9	275	41.7
	Brown	4	2	-		4	1.8	-	-	1	5	1	0.45	2	1	-	-	2	0.9	7	1.1
	Whitish pale	4	2	-		4	1.8	4	2	-	-	4	1.8	4	2	-	-	4	1.8	12	1.8
Test	X^2 and P-value																			34.8	***
Skin-color	White	109	54.5	17	85	126	57.2	100	50	4	20	104	47.2	82	41	10	50	92	41.8	322	48.8
	Yellow	24	12	-	-	24	10.9	41	20.5	6	30	47	21.3	57	28.5	6	30	63	28.6	134	20.3
	Grey	60	30	-	-	60	27.2	38	19	8	40	46	20.9	40	20	4	20	44	20	150	22.7
	Black	7	3.5	3	15	10	4.5	21	10.5	2	10	23	10.4	21	10.5	-	-	21	9.5	54	8.2
Tests	X^2 and P-value																			31.2	***

M= male; F= female; T=Total; N= Number of chicken exhibiting a particular qualitative character; X^2 = Chi square test; ***; significant at $P<0.01$

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Table 2 - Continued

Traits	Attribute	Agro-ecology																		Overall	
		Highland						Mid-altitude						Lowland							
		F		M		T		F		M		T		F		M		T			
		N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
Shank color	Yellow	88	44	12	60	100	45.5	80	40	15	75	105	47.7	86	43	16	80	102	46.4	297	45
	Grey	74	37	6	30	80	36.3	81	40.5	5	25	86	39	85	42.5	4	20	89	40.4	255	38.6
	Black	4	2	2	10	6	2.7	14	7	-	-	14	6.3	3	1.5	-	-	3	1.3	23	3.5
	White	34	17	-	-	34	15.4	25	12.5	-	-	25	11.3	26	13	-	-	26	11.8	85	12.9
Tests	$X^2$and P-value																			10.9	0.09
Feather distribution	Normal	198	99	20	100	218	99.1	198	99	18	90	216	98.18	183	91.5	18	90	201	91.36	635	96.2
	Nacked neck	2	1	-		2	0.9	2	1	2	10	4	1.8	17	8.5	2	10	19	8.6	25	3.8
Tests	$X^2$and P- value																			21.5	0.0001
Feather morphology	Normal	200	100	20	100	220	100	200	100	20	100	220	100	200	100	20	100	220	100	660	100
	Silky	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Test	$X^2$and P-value																				
Head shape	Flat plain	196	98	20	100	216	98.18	184	92	20	100	204	92.7	183	91.5	20	100	203	92.3	623	94.4
	Gutye	4	2	-	-	4	1.8	16	8	-	-	16	7.2	17	8.5	-	-	17	7.7	37	5.6
Tests	$X^2$and p-value																			8.9	0.01
Comb type	Single	78	39	2	10	80	36.3	156	78	13	65	169	76.8	159	79.5	19	95	178	80.9	427	64.7
	Double	42	21	4	20	46	20.9	8	4	2	10	10	4.54	12	6	-	-	12	5.45	68	10.3
	Pea	16	8	-	-	16	7.2	14	7	-	-	14	6.36	6	3	-	-	7	3.18	36	5.6
	Rose	64	32	14	70	78	35.45	22	11	5	25	27	12.27	23	11.5	1	5	23	10.45	129	19.4
Tests	X^2 and P-Value																			125.04	0.000
Ear-lobe color	Red	134	67	10	50	144	65.4	57	28.5	10	50	67	30.45	62	31	5	25	67	30.4	278	42.1
	White	28	14	-	-	28	12.7	96	48	5	25	101	45.9	82	41	5	25	87	39.54	216	32.7
	Yellow	34	17	10	50	44	20	38	19	5	25	43	19.5	46	23	10	50	56	25.4	143	21.7
	Grey	4	2	-	-	4	1.8	7	3.5	-	-	7	3.1	4	2	-	-	4	1.8	15	2.3
	Dark brown	-	-	-	-	-	-	2	1	-	-	2	0.9	6	3	-	-	6	2.7	8	1.2
Test	X^2 andP-value																			94.7	0.0001

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Quantitative trait measurements

Data on live body weight and linear body measurements of existing chicken ecotypes are widely used in the selection program (Mohammed et al., 2017). According to Tareke et al. (2018), live body weight and linear body measurement play an important role in genetic improvement and breed selection. Live body weight (kg) and other linear body measurements (cm) of indigenous chickens across the studied agro-ecologies are shown in Table 3.

Table 3 - Least squares mean (LSM) and (SE) of live body weight (kg) and other linear body measurements (cm) of indigenous chicken affected by agro-ecology, sex, and sex × agro-ecology interaction

Levels	N	BW	BL	CC	NL	TC	SL	HB
		LSM±SE	LSM±SE	LSM±SE	LSM±SE	LSM±SE	LSM±SE	LSM±SE
Overall	660	1.8±0.01	39.6±0.1	26.5±0.05	11.2±0.07	10.8±0.06	7.7±0.02	27.7±0.06
CV		9.5	4.07	2.9	9.6	10.5	5.22	3.66
R^2		0.66	0.40	0.73	0.56	0.60	0.39	0.61
Agro-ecology		**	**	**	**	**	**	**
Highland	220	1.7±0.01^a	39.6±0.13^a	26.6±0.09^a	10.9±0.09^a	10.7±0.09^a	7.8±0.03^c	27.8±0.12^a
Mid-altitude	220	1.8±0.01^b	40.5±0.11^b	27.2±0.09^b	11.6±0.07^b	11.8±0.07^b	7.7±0.02^b	27.8±0.10^a
Lowland	220	1.7±0.01^a	39.4±0.13^a	26.8±0.08^a	10.8±0.10^a	10.0±0.10^a	7.5±0.02^a	27.3±0.09^b
Sex		**	**	**	**	**	**	**
Male	60	2.1±0.02	42.0±0.19	29.0±0.18	12.9±0.09	13.2±0.16	8.2±0.08	29.7±0.2
Female	600	1.4±0.00	37.7±0.06	24.8±0.03	9.4±0.05	9.2±0.04	7.2±0.01	25.5±0.01
Sex × agro-ecology		**	**	**	**	**	*	*
Male, highland	20	2.1±0.02^a	42.3±0.15^a	28.8±0.22^a	13.1±0.13^a	13.2±0.24^a	8.7±0.09^a	30.2±0.17^a
Female, highland	200	1.4±0.03^a	37.3±0.10^a	24.5±0.04^a	9.8±0.08a	8.7±0.06^a	7.3±0.02^a	25.7±0.08^a
Male, mid-altitude	20	2.2±0.05^b	42.2±0.39^a	29.4±0.26^b	13.0±0.08^a	13.6±0.18^b	8.0±0.13^b	30.1±0.39^b
Female, mid-altitude	200	1.5±0.05^b	38.4±0.09^b	25.1±0.05^b	10.0±0.05^a	9.9±0.05^b	7.2±0.02^a	25.7±0.05^a
Male, lowland	20	2.0±0.03^c	41.5±0.24^b	28.9±0.27^a	12.5±0.22^b	12.8±0.20^c	7.9±0.14^b	28.9±0.27^c
Female, lowland	200	1.4±0.01^a	37.3±0.12^a	24.7±0.05^a	9.1±0.08^b	9.0±0.09^c	7.0±0.02^a	25.3±0.06^a

Table 3 - Continued

	N	SC	CL	CH	WW	WL	BkL	BkW	WS
		LSM±SE	LSM±SE	LSM±SE	LSM±SE	LSM±SE	LSM±SE	LSM±SE	LSM±SE
Overall	660	4.1±0.02	3.9±0.04	3.1±0.05	3.3±0.03	3.6±0.04	2.5±0.02	1.6±0.01	40.2±0.09
CV		10.72	22.82	37.78	18.43	24.65	12.48	16.17	3.48
R^2		0.45	0.66	0.58	0.73	0.66	0.10	0.11	0.42
Agro-ecology		**	**	NS	**	**	NS	NS	**
Highland	220	4.0±0.03^a	4.0±0.07^a	3.1±0.08^a	3.4±0.04a	3.7±0.06a	2.2±0.03a	1.6±0.01^a	40.2±0.12^a
Mid-altitude	200	4.2±0.03^b	4.0±0.07^a	3.2±0.07^a	3.7±0.06b	3.8±0.07a	2.3±0.02a	1.6±0.02^a	40.8±0.10^a
Lowland	220	3.9±0.03^a	3.7±0.06^b	2.9±0.07^a	3.4±0.05a	3.3±0.07b	2.3±0.02a	1.6±0.01^a	39.8±0.11^b
Sex		**	**	**	**	**	**	**	**
Male	60	4.7±0.09	5.4±0.17	4.5±0.18	4.7±0.14	5.1±0.15	2.3±0.05	1.7±0.02	42.2±0.17
Female	600	3.5±0.01	2.4±0.02	1.6±0.02	2.2±0.02	2.1±0.02	2.2±0.01	1.5±0.01	38.4±0.06
Sex × agro-ecology		*	*	**	**	**	NS	*	**
Male, highland	20	4.5±0.12^a	5.6±0.38^a	4.8±0.28^a	4.6±0.13^a	4.7±0.09^a	2.4±0.07^a	1.8±0.03^a	42.6±0.20a
Female, highland	200	3.4±0.02^a	2.5±0.03^a	1.6±0.04^a	2.1±0.03^a	2.3±0.04^a	2.2±0.02^b	1.5±0.01a	38.2±0.09a
Male, mid-altitude	20	4.9±0.17^b	5.4±0.32^a	4.4±0.39^b	5.1±0.22^b	5.6±0.29^b	2.2±0.07^a	1.6±0.03^b	42.5±0.09a
Female, mid-altitude	200	3.6±0.02^a	2.5±0.03^a	1.7±0.04^b	2.5±0.02^b	2.3±0.03^a	2.2±0.02^b	1.6±0.02^a	39.0±0.08b
Male, lowland	20	4.4±0.13^a	5.2±0.18^a	4.4±0.29^b	4.6±0.21a	4.9±0.29^a	2.3±0.07a	1.5±0.04^b	41.5±0.23b
Female, lowland	200	3.4±0.02^a	2.2±0.03^b	1.5±0.03^c	2.0±0.02a	1.8±0.03^b	2.3±0.02^b	1.5±0.01^a	38.0±0.10a

LSM=Least squares means; SE= Standard error; R^2=R-square; CV=Coefficient of Variation; Means with different superscripts within the same column and class are statistically different (at least *P*<0.05); Ns = Non –significant; * Significant at (P< 0.05); **significant at (P<0.01); BW=Body Weight; BL=Body Length; CC= Chest circumference; NL=Neck length; TC=Thigh circumference; SL=Shank length; HB= height at back; SC=Shank circumference; CL=Comb length; BkL= Beak length; BkW= Beak Width; WL= Wattle length; CH=Comb height; WW=Wattle width.

Effect of agro-ecology, sex and their interaction

Agro-ecology

Body weight (BW), body length (BL), chest circumference (CC), neck length (NL), thigh circumference (TC), shank length (SL), height at back (HB), shank circumference (SC), comb length (CL), wattle width (WW), wattle length (WL), and Wing Span (WS) showed a significant difference (P<0.05) among the studied agro-ecologies. However, beak length (BkL), beak width (BkW), and comb height (CH) were not significantly (P>0.05) different. Similarly, Melesse and Negesse (2011) indicated that significant differences were observed in CC, SL, NL, BL, WL, WS, WW, CL, and HB of local chicken ecotypes across all agro-ecologies of Tigray's central zone. The body weight of chickens (1.8±0.02 kg) in the mid-altitude was significantly higher (P<0.05) than chickens in the highland (1.7±0.01 kg) and lowland agro-ecologies (1.7±0.01 kg). The variation in body weight might be due to the existence of many strains, management practices, and production systems among the studied agro-ecologies. The mean body weight of chicken (1.8±0.02 kg in mid-altitude) was higher than Tareke et al. (2018), who reported that chickens reared in the Bale zone Oromia regional state weighed 1.1 kg and Assefa and Melesse (2018) who found that the overall mean body weight of indigenous chicken in Sheka zone was 1.68±0.2 kg. The

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mean body weight of chickens in the current study indicated that local chickens were heavier in the studied agro-ecologies. This implies they are more productive for carcass production. This agrees with there is strong correlation with meat yield and body weight as a proxy indicator of production (FAO, 2012). On the other hand, the CC values for highland, midland, and lowland were 26.6±0.09, 27.2±0.09, and 26.80±0.08 cm, respectively.

The result on CC was higher than the values (25.4±0.1 cm) of Tareke et al. (2018) for indigenous chicken in Bale zone. The wingspan values were 40.2±0.12, 40.8±0.10, and 39.8±0.11 cm in highland, mid-altitude, and lowland agro-ecologies, respectively, and the wattle width values were 3.4±0.04, 3.7±0.06, and 3.4±0.05 cm, in similar agro-ecologies respectively. The result on the wingspan was lower than the values (50.7±3.1 cm for males and 44.5±2.1 cm for females) of Assefa and Melesse (2018) for indigenous chickens in the Sheka zone.

Sex

Table 3 shows the average live body weight (kg) and other linear body measurements (cm) as affected by sex. The current results showed that sex had a significant (P<0.05) effect on body weight (BW), body length (BL), chest circumference (CC), neck length (NL), thigh circumference (TC), shank length (SL), height at back (HB), shank circumference (SC), comb length (CL), comb height (CH), wattle width (WW), wattle length (WL), wingspan (WS), beak length (BkL), beak width (BkW). The effect of sex on body weight and other body linear parameters found in this study agree with those of Melesse and Negesse (2011) for indigenous chicken in different Ethiopian zones, Assefa and Melesse (2018a,b) for Sheka indigenous chicken, and Tareke et al. (2018) for Bale indigenous chicken.

In all statistically analyzed linear body measurements, the male chicken was significantly higher (P<0.05) than its female counterpart; such variations might be attributed to the differential effects of testosterone in optimizing growth on muscle development and growth in general, as well as a more strong selection pressure on males than females (Islam et al., 2021). The lower body measurement values observed in this study for female chickens than for male chickens were also consistent with the findings of Fitsum (2015) who found that sexual dimorphism in chickens was manifested in a wide range of body attributes and across most breeds. This could be due to sex hormones, which may encourage males to build more muscles than girls.

Sex and agro-ecology interaction

The interaction of sex and agro-ecology had a significant effect (P<0.05) on SL, HB, SC, CL, BkW, BW, BL, CC, NL, TC, CH, WW, WL, and WS. However, they were not significant (P>0.05) for BkL. On contrary, Fitsum (2015) found that the interaction of sex and agro-ecology had no statistically significant (P>0.05) effect on BW and other linear body measurements of local chickens in the central Tigray zone. The significant variation in interaction between sexes and agro-ecology indicates the presence of distinct subgroups within the local chicken population. This diversity allows for genetic improvement both between and within sub-populations. The average body weights (BW) of male chickens in highland, mid-altitude, and lowland areas were 2.1±0.02, 2.2±0.05, and 2.0±0.03 kg, respectively. Males and female chickens were heavier at mid-altitude (2.1±0.02 and 1.5±0.04 kg) than the weight of chickens in highland (2.2±0.05 kg and 1.4±0.03 kg) and lowland (2.0±0.03 and 1.4±0.01 kg) agro-ecologies (Table 3) which corresponds to the finding of Hailu et al. (2018 a,b) who reported that the average body weight of male and female chicken in Guji zone was 2.1±0.05 and 1.5±0.02 kg, respectively. However, the result was higher than the values of Fitsum (2017) who indicated that the average live body weight of chicken in chicken midland and highland agro-ecologies were 1.36±0.02 and 1.36±0.03 kg, respectively.

Males' body length in highland, mid-altitude, and lowland agro-ecologies was 42.3±0.15, 42.2 ±0.39 and 41.5±0.24 cm, respectively, whereas for females about 37.3±0.10, 38.4±0.09, and 37.3±0.12 cm, were recorded in highland, mid-altitude, and lowland agro-ecologies, respectively. Chest circumference in male chickens was 28.8±0.22, 29.4±0.26, and 28.9±0.27 cm, and similarly, for females, 24.5±0.04, 25.1±0.05, and 24.7±0.05 cm were reported in highland, mid-altitude, and lowland agro-ecologies, respectively. The results on the measurements of the chickens' chest circumference and body length were consistent with those of Tadele et al. (2019) who found average body lengths of 41±0.11 and 37.4±0.08 cm for males and female chickens, respectively. Furthermore, Hailu et al. (2018) revealed that male and female indigenous chickens in the Guji zone had chest circumferences of 27.6±0.01% and 25.3±0.06%, respectively, and that the body length of indigenous chickens was 41.1 cm.

Neck length showed 13.1±0.13, 13.0±0.08, and 12.5±0.22 cm for males and 9.8±0.08, 10.0±0.05, and 9.1±0.08 cm for females in highland, mid-altitude, and lowland agro-ecologies, respectively. The shank length in highland, mid-altitude, and lowland agro-ecologies for male chickens was 8.7±0.09, 8.0±0.13, 7.9±0.14 cm, respectively, while for females it was 7.3±0.02, 7.2±0.02, and 7.0±0.02 cm, respectively. The current finding is similar to the report of Alebachew et al. (2019) who showed that shank length was 8.1±0.89 and 6.8±0.94 cm for males and females, respectively, while neck length was 12.6±3.2 and 10.8±2.13 cm for males and females in Benshangul Gumuz district. The findings were also in line with Tadele et al. (2019), who found that the average shank length of indigenous chickens in North Gondar was 8.1 cm and 7.49 cm in the Keffa zone (Tadele et al., 2019). The male wingspan was 42.6±0.20, 42.5±0.09 and 41.5±0.23 cm, while for the female it was 38.2±0.09, 39.0±0.08 and 38.0±0.10 cm, in Highland, midland and lowland agro-ecologies, respectively. The current finding was lower than Getachew and Negassi (2016) who stated that the wingspan of indigenous chickens in the study region was 70.34 cm for males and 60.87 cm for females, respectively in Bench-Maji zone. However, Guni and Katule (2013) found similar results for hens at 47.6 cm raised in Tanzania's Southern Highlands. The results of this study's wingspan were equivalent to those reported by Tadele et al. (2019) in the Keffa and North Shewa zones. In the current study, indigenous chickens had wingspans of 38.8 cm and 38.45 cm.

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Body weight of young chickens reached for market

The average body weight of mature indigenous chickens at marketable weight is shown in Table 4. There was a significant (P<0.05) difference in body weight between the sexes and across the study agro-ecologies. The average body weight of local adult chicken was 1.45±0.02 kg, which was higher than Aklilu et al. (2013) who reported values for Ethiopia's Horro and Jarso districts (1.29 kg and 1.12 kg, respectively), Yami and Dessie (1997) who reported values for Ethiopia's central highlands (1.04 kg) and Mogesse (2007b) who reported values for northwest Ethiopia (847.77 g). Similar to the current findings, Hailu et al. (2018 a,b) found higher average body weight results for indigenous chicken populations in Guji zone than the current finding. The body weight of chickens reaching market age in the midland was significantly higher (P<0.05) than in the lowland and highland agro-ecologies (P<0.01), implying that chickens in the mid-altitude weigh better at market age and/or reach market age at an earlier age than those in the lowland and highland agro-ecologies. The sex of chicken had a significant influence on body weight at market age (P<0.05) in this study. These might be due to management or environmental factors, within this age group; the average body weight for both sexes was 1.71±0.01 kg for men and 1.45±0.02 kg for females, respectively. The current finding for both sexes is in accordance with Agarwal et al. (2020) for native chickens of the Chotanagpur plateau of Jharkhand. However, Sanka and Mbaga (2014) found lower results for Tanzanian local chicken reared under intensive and semi-intensive production systems than the current research output. The result pertaining to body weight was higher than Padhi (2016), for indigenous chicken ecotypes. Sexual dimorphism is between the traits where the male chicken has a higher body weight when compared to the female chicken (Sanka and Mbaga, 2014). Body weight of both sexes at market age was significantly affected by agro-ecology and sex interaction (P<0.01). Males in the mid-altitude zone (1.8±0.05 kg) weighed more than those in the lowland zone (1.7±0.02 kg) and the highland zone (1.6±0.03 kg). Females in the mid-altitude, lowland, and highland agro-ecologies, respectively, weighed 1.5±0.01, 1.5±0.04, and 1.4±0.02 kg at market age.

Body weight prediction

The use of regression equations to predict animal weight from other easily obtained linear body parameters is critical in animal selection and marketing (Taye et al., 2016). The precision of functions used to forecast live weight or growth parameters from live animal data helps livestock producers to save a lot of money and time (Rojo-Gimeno et al., 2019). Bodyweight is a crucial measurement in poultry production since it is used to determine not only growth and feed efficiency but also economic and management choices (Dahloum et al., 2016).

However, a scale may not be provided in other situations. Scientists have developed prediction models to estimate live weight using linear body measurements due to practical problems in measuring live weight at the field level (Dahloum et al., 2016). Multiple regression models are excellent for forecasting animal body weight. However, because of the large number of predicted variables in the model, their biological interpretation might be misleading (Mendeş, 2009).

Multiple regression models were constructed for the estimation of body weight (BW) from other body linear measurements. Body length (BL), Chest circumference (CC), Neck length (NL), thigh circumference (TC), Back height (HB), Shank length (SL), Shank circumference (SC), Wingspan (WS), Comb length (CL), Comb height (CH), Wattle width (WW), Beak length (BL), and Beak width (BW) were all the measurements. Stepwise regression was used to choose independent variables for both sexes in each agro-ecology by entering all of the following features except BkL one at a time for males and females. Due to its bigger contribution to the model than other variables, chest circumference was consistently selected and put into the model in step one of stepwise regression among sex and agro-ecologies. This conclusion was in line with Liyanage et al. (2015) who found significant connections between body weight and every linear characteristic using regression analysis, with chest circumference and shank length being the strongest predictors of live weight in Sri Lankan village chickens. In the second phase of stepwise regression, two independent variables were chosen to be included in the model, three independent variables in the third step, and so on. The process of adding significant (P<0.05) and best among the rest of the variables to the model proceeded in phases until no other variable matched the P<0.05 significance threshold for inclusion. A selection of variables was used in each step after analyzing all variables to see whether any should be eliminated at that phase. For both sexes, the number of variables included in each stage, parameter estimates, and their contribution in terms of coefficient of determination (R2), root mean square error (MSE), Mallows C parameters C (p), Alkaike's Information Criteria (AIC), and Schwarz Bayesian Criteria (SBC) as shown in (Table 5). The coefficient of determination (R2) shows the percentage of total variability that the model accounts for.

In males, chest circumference was the first variable to explain more variation than other factors (87–90%), whereas BL was the first variable to explain more variation in females (59–80%).In line with the current study, Yakubu et al. (2009) and Ajayi et al. (2012) reported that body length (BL) was the most important contributor to variation in body weight in normal feathered Nigerian indigenous chickens. CC explained more variance for males (85% to 91%) than other variables under mid-land agro-ecology, but BL explained more variance for females (54% to 73%) than other body linear measurements. Similarly, in lowland agro-ecology, for males, CC explains (80 to 84 %) more variance for males than the remaining factors in lowland-agro-ecology, but BL for females had the first variable (69 to 80 %) to explain variance. In general, the R2 value of CC for males was lower in lowland agro-ecology than in the highland and mid-altitude agro-ecologies, but the R2 value of BL for females was lower in mid-altitude agro-ecology than in both highland and lowland agro-ecologies.

In highland altitude, y =-1.02 + 0.10 CC for male and y =-1.15 + 0.07BL + 0.09 NL for female, y =-1.06+ 0.11 CC for male and y =-0.76+ 0.04 BL + 0.06 TC for female in midland, and similarly, y =-0.90 + 0.10 CC for lowland male and y =-1.33 + 0.07 BL. As a result, CC for males and BL for females was the best predictor for predicting chicken body weight above other factors.

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Table 4 - Least squares means (LSM) ± standard error (SE) of live body weight (kg) of indigenous chickens reached for market the main effect of agro-ecology, sex, and sex by agro-ecology interaction

Levels	Number	Body Weight
Overall	180	1.6±0.01
R^2	180	0.36
CV	180	11.59
Agro-ecology		**
Highland	60	1.52±0.02[a]
Midland	60	1.66±0.04[b]
Lowland	60	1.56±0.1a
Sex		**
Male	120	1.71±0.01
Female	60	1.45±0.02
Sex × agro-ecology		**
Male; Highland	40	1.6±0.03[a]
Male; Mid-altitude	40	1.8 ±0.05[b]
Male; Lowland	40	1.7±0.02[c]

R^2=R-square; CV=Coefficient of Variation; Means with different superscripts within the same column and class are statistically different (at least P<0.05); **significant at (P<0.01).

Table 5 - Multiple regressions between body weight and other linear measurement for both sexes in study agro-ecology

Agro-ecology	Sex	Model	Intercept	β_1	β_2	β_3	β_4	β_5	R^2	C(P)	AIC	Root MSE	SBC
Highland	Male	CC	-1.02	0.10					0.87	9.18	-125.6	0.04	-123.6
		CC+TC	-0.68	0.07	0.03				0.90	1.57	-129.6	0.03	-126.6
	Female	BL	-1.26	0.07					0.59	230.3	-918.1	0.10	-911.5
		BL+NL	-1.15	0.04	0.09				0.73	81.3	-1003.5	0.08	-993.6
		BL+NL+SC	-1.24	0.04	0.08	0.07			0.76	50.5	-1026.9	0.07	-1013.7
		BL+NL+SC+HB	-1.55	0.03	0.07	0.06	0.02		0.79	30.2	-1044.0	0.07	-1027.7
		BL+NL+SC+HB+WL	-1.47	0.03	0.06	0.07	0.02	-0.02	0.80	21.7	-1052	0.07	-1032.2
Mid-altitude	Male	CC	-1.06	0.11					0.85	2.3	-99.20	0.07	-97.2
		CC+BL	-0.59	0.06	0.07				0.90	-1.4	-104.8	0.06	-101.9
		CC+BL+SL	-4.19	0.05	0.09	0.08			0.91	-0.5	-104.6	0.06	-101.4
	Female	BL	-0.78	0.05					0.54	141.6	-1058.5	0.07	-1051.9
		BL+TC	-0.76	0.04	0.06				0.67	49.6	-1121.7	0.06	-1111.8
		BL+TC+NL	-0.65	0.03	0.05	0.04			0.73	9.33	-1157.8	0.05	-1144.6
Lowland	Male	CC	-0.90	0.10					0.80	7.77	-109.6	0.06	-107.7
		CC+TC	-0.87	0.08	0.03				0.84	4.82	-112.3	0.05	-109.3
	Female	BL	-1.33	0.07					0.69	110.1	-980.5	0.08	-973.9
		BL+NL	-0.95	0.05	0.04				0.78	18.4	-1051.7	0.07	-1041.8
		BL+NL+HB	-1.45	0.04	0.04	0.03			0.80	5.23	-1064.5	0.06	-1051.3

R^2=R-square; MSE=Mean square of error; C(p)=Mallows C parameters; AIC =Alkaike's Information Criteria; SBC =Schwarz Bayesian Criteria

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CONCLUSION

The qualitative and quantitative features of indigenous chicken ecotypes showed significant phenotypic variation among sex and across agro-ecologies. The existence of significant genetic variability in indigenous chickens is supported by the large diversity of indigenous chicken phenotypes. The current study was one of the steps taken to document the chicken ecotype in the study area. Thus, the information could provide a better direction for developing a breeding plan for the improvement and conservation of indigenous chicken ecotypes. To improve the standardization of phenotypic descriptors, conservation, and genetic utilization, an in-depth molecular study is required to verify the level of genetic heterogeneity and relationship among indigenous local chicken ecotypes.

DECLARATIONS

We declare that this research work is original and has not been published elsewhere.

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Authors' Contribution

All authors contributed equally in conducting and writing the research

Conflict of interest

There are no conflicts of interest declared by the authors.

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THE IMPORTANCE OF THE FEEDING LEVELS AND ADEQUACY ON THE MEAT QUALITY AND PRODUCTIVITY PERFORMANCE OF CROSS-BRED BULLS

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RESEARCH ARTICLE

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ABSTRACT: The study aimed to determine the effect of the level and adequacy of feeding on the dynamics of live weight and meat productivity of Simmental × Holstein bulls with a close blood relationship (87.5%) for the Holstein breed. Three groups of bulls with 87.5% Holstein heredity in the genotype were formed, out of which two groups were experimental and one group served as a control group. The bulls of the control group received a diet compiled according to detailed standards, and their analogs from the experimental groups had a diet exceeding the norm by 10 and 20%, respectively. During the entire growing period, the bulls of the 1st experimental group consumed 3,564 energetic feed units (EFU) and 362.5 kg of digestible protein (DP); the 2nd experimental group consumed 3,875 EFU and 394.3 kg DP; and the control group consumed 3,245 EFU and 329.9 kg DP, respectively. In terms of carcass yield, bulls of the first and second experimental groups outperformed the ones from the control group by 2.41% and 3.92%. With an increase in the level of feeding in experimental bulls, fat deposition accelerated; thus, the meat of bulls of the experimental groups contained 2.13% and 2.54% more fat than in animals of the control group. At the same time, the protein content in the meat of experimental bulls decreased by 0.15 and 0.22% in comparison with the control group. The energy value of 1 kg of meat of experimental bulls was higher by 0.77-0.90 MJ. It can be concluded that a possible increase in the meat productivity of bulls-crossbreeds of the Holstein breed with a close blood relationship and the determination of planned indicators of animal growth should be taken into account when developing breeding programs.

Keywords: Breed, Bulls, Carcass yield, Genotype, Live weight.

INTRODUCTION

The use of Holstein bulls for cross-breeding with local cattle breeds in almost many countries with developed dairy cattle breeding is one of the promising fields for increasing their milk productivity and udder technology (Tuzov et al., 2018; Rodríguez-Bermúdez et al., 2019). At the same time, studies indicate that the meat productivity of young animals decreases in crossbreeds which obtained for propose of milk yield (Bown et al., 2016). However, the analysis shows that the results obtained by different researchers are contradictory, as the observations were conducted on animals of different body types and different levels of feeding (Pfuhl et al., 2007; Venkata Reddy et al., 2015; Velmatov et al., 2018).

The formation of meat productivity and the carcass yield in cattle depends on the rearing quality of young animals (Hozáková et al., 2020; Lamanov et al., 2020). Numerous studies have established that with proper organization of breeding, and in re results stated that animals can be fatten faster with less feed consumption, due to optimum nutritional management (Wanapat et al., 2015; Hozáková et al., 2020; Lamanov et al., 2020). Currently, dairy and double-proposed breeds show the same gains as the cattle of meat breeds, in young age (Hietala and Juga, 2017; Berry et al., 2019). This is especially important in the first months of postnatal development since this phase largely determines subsequent productivity (Hietala and Juga, 2017; Berry et al., 2019).

In Russia, almost all farms with different levels of feed availability and located in different natural and climatic conditions have mainly the Holstein breed (Sidorova, 2016). The analysis of the production situation of numerous farms shows that the problem of full-fledged feeding is quite urgent (Bostanova et al., 2021; Semkiv, 2021). In this regard, In the breeding program for meat production, dietary energy levels is a key factor in nutritional management, and it should be controlled with any cross-breeding programs, especially for meat propose (Osadchuk et al., 2017; Velmatov et al., 2018).

Therefore, the objective of present study was to evaluate the dynamics of live weight and meat productivity of cross-bred Simmental × Holstein young animals of the third generation with a different energy level and amount of feed.

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MATERIALS AND METHODS

Ethical regulation

In the course of the current study all procedures were conducted in accordance with the Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes and approved by institutional ethical review committees in Mordovia Research Institute of Agriculture and Ogarev Mordovia State University).

Experimental design

At the Agrosoyuz LLC of the Ruzaevsky district of the Mordovian Republic (Russia), three groups of bulls with 87.5% of Holstein heredity in the genotype were formed: two experimental groups and one group serving as a control variant, with 20 heads in each group. The bulls were selected according to the principle of analog pairs, taking into account the genotype and live weight. When compiling the diets for the control group bulls, we used the recommendations of the L.K. Ernst Federal Research Center for Animal Husbandry (VIZh, 2021; Marinchenko, 2021), and the bulls from the experimental groups received 10% or 20% more nutrients, in comparison with control.

To study the dynamics of the live weight of bulls, individual weighing was assayed at birth, and 3, 6, 9, 12, 15, and 18 months of age. The average daily, absolute, and relative gains in live weight were determined. To study the meat productivity of steers, we used the methodological recommendations of the All-Union Academy of Agricultural Sciences (VASKhNIL, 2019), and the All-Russian Research Institute of Medical Device Industry (Levantin et al., 2019). The slaughter of bulls was carried out at the Ichalkovsky meat processing plant of the Republic of Mordovia for 5 animals from each group. During the slaughter, the following indicators were taken into account: live weight (removable and pre-slaughter), carcass weight, fat weight, slaughter yield of carcass and fat (kg, %), internal organ weight, fresh skin weight

When studying the chemical composition of meat, a general sample of meat was used. To determine the content of protein, fat, and ash in meat, the following methods were used:

- Moisture content by drying the sample weight to a constant weight at a temperature of 105±2C;
- Protein content by the method of determination of total nitrogen by the Kjeldahl method (Kirk, 1950) in combination with isometric distillation in Conway's vessels;
- Fat content by extracting the dry sample weight with ether in the Soxhlet apparatus;
- The content of mineral substances (ash) by dry mineralization of samples in a muffle furnace at a temperature of 450-600 C according to the VIZh method (Levantin et al., 2019).

In present experiments, the first feeding of calves with colostrum was performed during the first 30 minutes after birth. The amount of colostrum consumed on the first day of life was 4-5 kg. At the same time, the health status of the calf was taken into account. During the dairy period, its tried not to overload the developing digestive system, to avoid digestive disorders, and also to achieve the necessary level of growth. Young calves from the age of 3 days received dry food (high-fiber) in the form of starter compound feed and whole grains of oats and corn in equal proportions. The use of starter feed and grain feeds makes it possible to transfer calves to coarse feed at an earlier time, which reduces milk consumption and labor costs and allows obtaining a developed calf capable of eating a large amount of vegetable feed. The size of granules and grains, and their physical characteristics are of no small importance for the correct starter feed and grain feed. The rough texture of the feed is preferable for the calf. In this connection, the consumption of dry matter increases with the inclusion of whole or slightly flattened corn, but not ground corn.

Statistical analysis

The data obtained in the course of the study were processed by the method of variation statistics proposed by SPSS software, with ANOVA method (P value in 0.05) (George and Mallery, 2019). The reliability of the indicators was assessed by Student's t-test.

RESULTS AND DISCUSSION

The feed of the farm's production was introduced into the diet of bulls, containing alfalfa hay, alfalfa haylage, corn silage and concentrates. It should be noted that hay and haylage are harvested in the budding phase of plants, and corn silage is harvested in the wax ripeness phase.

During the entire growing period, the bulls of the control group consumed 3245 energetic feed units (EFU) and 329.9 kg of digestible protein (DP) per head, the bulls of the first experimental group consumed 3564 EFU and 362.5 kg of DP, and the bulls of the second experimental group consumed 3875 EFU and 394.3 kg of DP, respectively. One EFU contained 101.7 g of DP (Table 1).

Intensive breeding following established feeding standards had a significant impact on the dynamics of the live weight of experimental animals (Table 2). Thus, at the age of three months, experimental bulls reliably outperformed the ones from the control group in live weight by 7.9 kg (8.1%; P≤0.01) and 10.8 kg (11.0%; P≤0.001). At six months of age, the difference increases to 16.4 kg (9.4%; P≤0.001) and 22.5 kg (12.9%; P≤0.001), at nine months of age by 17.9 kg (7.1%; P≤0.01) and 33.1 kg (13.1%; P≤0.001) at twelve months of age by 26.2 kg (8.2%; P≤0.01) and 55 kg (17.2%;

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P≤0.001), at fifteen months of age 33.6 kg (8.7%; P≤0.01) and 67.2 kg (17.5%; P≤0.001) and at eighteen months of age by 39.5 kg (8.6%; P≤0.001) and 73.8 kg (16.4%; P≤0.001).

When studying the dynamics of average daily gains, the unequal intensity of the weight gain in bulls was established. Differences in average daily gain were observed from birth to three months of age. The advantage of bulls of the experimental groups in this age period was 100 g (15.3%; P≤0.01) and 114.6 g (17.6%; P≤0.01)

From three to six months of age, the advantage of bulls of the experimental groups remained and amounted to 94.4 g (11.1%;) and 130.5 g (15.4%). A similar pattern was observed from six to nine months of age. Significant differences were noted between the bulls of the control and the second experimental group in the age period from nine to twelve months of age, equaling 243.9 g (32.1%; P≤0.001) (Table 3).

For the entire period, from birth to 18 months of age, the bulls of the second experimental group were characterized by a large average daily gain, so the difference between the bulls of the control group and the second experimental group was 136 g (17.9%; P≤0.05), and between the bulls of the control and the first experimental group, it equaled 73.6 g (9.7%; P≤0.05). When studying meat productivity, a control slaughter of bulls was carried out at the age of 18 months, when five heads from each group were slaughtered (Table 4).

Table 1 - Feed consumption by animals of experimental groups from birth to 18 months of age

Groups / Indicator	Control	Experimental	
		1st	2nd
Milk, kg	270	295	325
Milk replacer, kg	20	22	24
Prestarter feed, kg	45	57	75
Starter feed, kg	176	200	230
Hay, kg	239	197	177
Haylage, kg	4,010	4,183	4,295
Silage, kg	1,892	2,237	2,338
Straw, kg	305	305	305
Concentrates, kg	691	760	881
Energetic feed units (EFU)	3245	3564	3875
Digestible protein (DP), kg	329.9	362.5	394.3
DP content in 1 EFU	101.7	101.7	101.7

Table 2 - Dynamics of live weight of bulls, kg (X ± Sx)

Groups / Age, months	Control	Experimental	
		1st	2nd
At birth	39.7±0.57	39.2±0.55	40.0±0.49
3	97.6±1.80	105.5±1.62**	108.4±1.63***
6	173.9±3.54	190.3±4.01***	196.4±2.92***
9	251.1±4.93	269.0±3.68**	284.2±3.92***
12	319.5±5.47	345.7±5.44**	374.5±4.49***
15	384.4±7.92	418.0±5.25**	451.6±4.85***
18	450.9±8.21	489.9±6.02***	524.7±5.32***

*P≤0.05, **P≤0.01, ***P≤0.001 data are reliable.

Table 3 - Dynamics of the average daily weight gain of bulls, g (X±Sx)

Groups / Age, months	Control	Experimental	
		1st	2nd
0-3	643.3±25.45	737.2±22.78	760.0±23.61
3-6	847.8±39.34	942.2±48.87*	978.3±40.21*
6-9	857.2±47.27	873.9±48.62*	975.0±28.71*
9-12	760.0±39.94	852.2±43.79*	1,003.9±36.85***
12-15	721.1±99.58	803.9±49.94	856.1±58.87
15-18	738.9±86.37	798.9±61.73	812.2±47.47
0-18	761	834.6	897

*P≤0.05, **P≤0.01, ***P≤0.001 data are reliable.

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Table 4 - Slaughter indicators of bulls, (X±Sh)

Indicator / Groups	Control	Experimental	
		1st	2nd
Pre-slaughter live weight, kg	427.0±2.24	454.8±4.38	492.8±5.28
Hot carcass weight, kg	230.4±1.85	256.4±2.11***	282.5±3.08***
Carcass output, %	53.96±0.45	56.37±0.29***	57.88±0.86***
Internal fat weight, kg	8.46±0.77	8.90±1.01	10.10±1.15
Fat output, %	1.98±0.22	1.95±0.27	2.05±0.27
Slaughter yield, %	55.93±0.49	58.32±0.24***	59.93±0.55***

*P≤0.05, **P≤0.01, ***P≤0.001 data are reliable.

Before slaughter, the fatness of the bulls was evaluated and was recognized as the highest in all animals. The carcasses obtained during slaughter were assigned to the first category. During the visual assessment of the carcasses of bulls of the second experimental group, they were distinguished by a large development of subcutaneous fat. In the bulls of the control group, this feature was less pronounced. Intensive feeding has left its mark on the formation of the muscles of the animals' torso. The bulls of the second experimental group had well-developed legs and a developed muscled trunk.

The heaviest carcasses were obtained from bulls of the second experimental group. Their advantage over the control group was 52.1 kg (22.6%; P≤0.001), and bulls of the first experimental group outperformed animals from the control group by 26.0 kg (11.3%; P≤0.001). In terms of carcass yield, bulls of the first and second experimental groups outperformed the ones from the control group by 2.41 and 3.92% (P≤0.001), and a similar pattern was observed in the slaughter yield. This finding is in agreement with Cattelam et al. (2018) who reports high-grain diets increase energy and carcass yield of fattening cows. Also its in according to Qiu et al. (2020) who stated high-energy diets in Holstein steers improves growth rate of animals and carcass yield. Also, in this regard, Nogalski et al. (2018) showed that During the fattening period, a higher proportion of concentrate (higher energy) in the ration contributed to higher feed intake, higher feed efficiency and more desirable carcass characteristics, which it observed in present study for cross-bred animals.

There were no significant differences in the output of internal fat between the animals of the first experimental group and the control group. This finding in in agreement with Jones et al. (1985) who reported no differences in the proportion of carcass weight relative to empty body weight for animals fed concentrate or forage diets. The bulls of the first and second experimental groups outperformed the ones from the control group by 0.44-1.64 kg (5.2-19.4%) (Table 4). Concerning the skin weight, one could note the superiority of the bulls from the experimental groups. Thus, the bulls of the first and second experimental groups outperformed the animals from the control group by 2.66 kg (8.4%; P≤0.01) and by 7.8 kg (24.7%; P≤0.001). The absolute weight of the head, front and hind legs in the control group bulls were lower than in the experimental groups.

Concerning the weight of internal organs and by-product yield one can note the advantage of the second experimental group bulls over the control group animals: the heart weight exceeded that of the control group by 0.7 kg (45.4%; P≤0.001), the lung weight by 0.96 kg (50.3), the liver weight by 1.68 kg (32.4%; P≤0.001), and the kidney weight by 0.32 kg (P≤0.05) (Table 5). As a result of the carcass dissection, intergroup differences in the morphological composition were revealed.

Table 5 - The weight of organs and tissues, kg (X±Sh)

Indicator / Groups	Control	Experimental	
		1st	2nd
Skin	31.60±0.61	34.26±0.98**	39.40±0.54***
Head	15.74±0.29	15.90±0.30	16.26±0.41
Tongue	0.78±0.04	0.80±0.06	1.04±0.16
Front legs	4.66±0.13	4.70±0.13	5.32±0.08
Hind legs	5.16±0.19	5.32±0.24	6.50±0.24
Testes	0.78±0.04	0.85±0.04	0.82±0.02
Heart	1.54±0.05	1.80±0.09	2.24±0.08***
Lungs	3.42±0.10	4.38±0.33	5.14±0.44***
Liver	5.12±0.13	5.66±0.23	6.80±0.31***
Kidneys	1.20±0.08	1.34±0.12	1.52±0.09*
Spleen	0.72±0.03	0.86±0.05	1.08±0.12

*P≤0.05, **P≤0.01, ***P≤0.001 data are reliable.

The carcasses of the experimental groups differed by the greater absolute flesh weight. Their advantage compared to the control group was 24.0 kg (13.4%; P≤0.001) and 43.8 kg (24.4%; P≤0.001). The absolute bone weight was lower in

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the control group compared to the experimental ones by 3.2 kg (7.4%) and 6.8 kg (15.8%) (Table 6). There were no significant differences in the weight of cartilage and tendons between the groups of animals. After dissecting, it was found that in the half-carcasses of experimental bulls, the flesh weight, expressed as a percentage of the carcass weight, was greater, and the bone weight was less, therefore, their meat index was 0.13-0.39% higher compared to the control group. The study of the chemical composition of the general sample showed that the dry substance content was the highest in the meat of bulls of the experimental groups, being 1.95% (P≤0.01) and 2.27% (P≤0.01) higher than the meat of bulls of the control group.

Table 6 - Morphological composition of bull carcasses, (X±Sh)

Group	Carcass weight, kg	Flesh		Bones		Cartilage and tendons		Meat index, %
		kg	%	kg	%	kg	%	
Control	230	179.6	78.08	43.0	18.69	7.4	3.22	4.17
1st experimental	257.6	203.6***	79.03	46.2	17.93	7.76	3.02	4.30
2nd experimental	282.0	223.4***	79.22	49.8	17.66	9.2	2.98	4.56

*P≤0.05, **P≤0.01, ***P≤0.001 data are reliable.

Table 6 - Chemical composition of the general meat sample

Indicator / Groups	Control	Experimental	
		1st	2nd
Moisture	68.51±0.36	66.56±0.39	66.24±0.33
Dry matter	31.49±0.36	33.44±0.39**	33.76±0.33**
Protein	19.45±0.10*	19.30±0.39	19.23±0.09
Fat	11.18±0.36	13.31±0.62**	13.72±0.31**
Ash	0.86±0.02	0.83±0.02	0.80±0.02
The protein and fat ratio	1:0.57	1:0.69	1:0.71
Caloric content of 1 kg of meat, MJ	4.16	4.93	5.06
Protein content in the carcass, kg	34.8	39.2	43.0
Fat content in the carcass, kg	20.0	27.0	30.6

*P≤0.05, **P≤0.01, ***P≤0.001 data are reliable.

With an increase in the level of feeding in experimental bulls, fat deposition accelerated; thus, the meat of bulls of the experimental groups contained 2.13% (P≤0.01) and 2.54% (P≤0.01) more fat than in animals of the control group. Present findings are in agreement with Hornick et al. (1998) who stated higher dietary concentrations in energy and protein, had higher carcass contents (connective and adipose tissue) whereas these animals had lower meat-fat content. Honig et al. (2020) showed that carcass fat increased during growth primarily due to feeding high-energy diet. This may be occurred due to expense of bone and subsidiary muscle tissue in this period. Also, Omarov et al. (2017) suggested similar feeding method (high level of energy levels with special oil seeds) for improve meat quality and develop marble beef production in Russian conditions.

At the same time, the protein content in the meat of experimental bulls decreased by 0.15 and 0.22% (P≤0.05) in comparison with the control group. This finding is in agreement with De Smet et al. (2000) which increased dietary concentration of some major nutrients can change energy/protein ratio in beef, whereas increased daily weight gain during different stages of the fattening period. Therefore, the ratio of protein and fat in experimental animals was not the same. These differences in energy/protein ratio in meat, due to high-energy diets have been reported and stated by Cantalapiedra-Hijar et al. (2018), Mwangi et al. (2019), and Maresca et al. (2019).

Thus, in animals of the control group, it was 1:0.57, in the first experimental group 1:0.69, and the second experimental group 1:0.71. The meat of the bulls of the experimental groups was more caloric than the meat of the animals of the control group. The energy value of 1 kg of meat of experimental bulls was higher by 0.77-0.90 MJ (Table 8). Present findings (Table 8), are in agreement with Siddiki et al. (2021) in their practice on buffaloes. Based on the conducted study, it should be noted that for Holstinized animals it is necessary to create optimal conditions for feeding and keeping, which makes it possible to identify the genetically determined productivity potential as much as possible while increasing the efficiency of their breeding.

An increase in the energy nutritional value of diets by 10-20% makes it possible to increase the live weight by 39.0-73.8 kg, to get bulls with a live weight of 489-524 kg. The relative growth rate, which reflects the intensity of the growth of bulls, shows that with an increased type of feeding, the growth rate increases at a young age. From birth to 18 months of age, the bulls of the second experimental group were characterized by a large average daily increase. Thus, the difference

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between the bulls of the control group and the second experimental group was 136 g (17.9%; P≤0.95), and between the bulls of the control and the first experimental group, it equaled 73.6 g (9.7%; P≤0.95).

At the same time, the quality characteristics of the carcass were improved. The ratio of flesh and bones with an increase in the feeding level by 20% was 4.56, with an increase in the feeding level by 10%, the ratio was 4.30 and in the control group, it was 4.17. At the same time, each carcass contained 73.6 kg, 66.1 kg, and 54.6 kg of protein and fat, respectively, the difference between the second experimental group and the control group was 34.7%, and between the first experimental group and the control group, it was 21.1%. At the same time, the differences in live weight before slaughter were 15.4% and 6.5%. With an increase in the level of feeding, the yield of valuable cuts, the yield of meat, protein, and fat increased.

CONCLUSION

The study data demonstrate the existence of early maturing animals which, with an increase in the level of feeding, can intensively increase live weight at a young age. The feeding system and the nature of growth had a significant impact on the formation of meat productivity and the quality indicators of the meat obtained from them. When developing breeding programs, it is necessary to take into account the possible increase in meat productivity of Holstein crossbred bulls with a close blood relationship and the determination of planned indicators of animal growth. All this should be taken into account when developing the technology of raising young animals for meat and determining the quality of products.

DECLARATIONS

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Authors' contribution

All the authors had similar roles in conceptualizes investigations, data collection, data analysis, laboratory analysis and manuscript writing.

Conflict of interests

The authors have not declared any conflict of interests.

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EFFECT OF DIFFERENT UNSATURATED FATTY ACIDS SOURCES ON *IN VITRO* FERMENTABILITY AND DIGESTIBILITY OF RATION IN DAIRY CATTLE

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RESEARCH ARTICLE

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ABSTRACT: Supplementation of oil rich in unsaturated fatty acids (FAs) such as canola, soybean, and palm oils improved the quality of milk fatty acids. However, the unprotected unsaturated oil might impair rumen fermentation, feed, and fiber digestibility. A study was conducted to determine the best type of oil supplementation (factor A) including canola (A1), soybean (A2), or palm (A3) and level oil supplementation (factor B) including B0 = 0%, B1 = 1%, B2 = 2% or B3 = 3%) on the *in vitro* feed fermentation and digestibility. The study used a 3 x 4 factorial block design. Two-stages were used to measure the pH, ammonia (NH3), volatile fatty acids (VFAs), protozoal number, dry matter (DMD), organic matter (OMD), neutral detergent fiber (NDFD), and acid detergent fiber (ADFD), digestibility. The results showed that oil type did not significantly influence the fermentability (pH, NH3, VFAs, and protozoa) and feed's digestibility (DMD, OMD, NDFD, and ADFD) but oil level influence the fermentability and digestibility significantly. In addition, an increase above 1% in oil levels reduced protein fermentability, protozoal number, DMD, and OMD, but increased VFAs. It is concluded that the addition of unprotected canola, soybean, or palm oil in dairy cattle ration could be applied in a concentration not more than 1%.

Keywords: Canola oil, Milk fatty acid, Palm oil, Ration, Soybean oil.

INTRODUCTION

Milk fatty acids (FAs) have become increasingly important in consumer awareness due to their link with human's health (AbuGhazaleh, 2008; Bauman et al., 2006; Despal et al., 2021a). They have positive and negative effects (Chen and Liu 2020). About 400 FAs were identified in milk with 4 to 26 carbons chain length with different degrees, positions, and configurations of unsaturation (Amores and Virto, 2019). However, not more than 30 FAs can be detected in tropical dairy cattle milk (Anzhany et al., 2021; Despal et al., 2021a; Riestanti et al., 2021) due to their small quantity. Saturated FA increases the risk of some diseases (Despal et al., 2021b). Actually, not all SFA harm human's health. Only the C12:0, C14:0, and C16:0 were considered unhealthy. While the C4:0, C6:0, C8:0, C10:0, C18:0 have been reported to have beneficial effects (González-Martín et al., 2020). In contrast, polyunsaturated fatty acids (PUFAs) have a beneficial effect since they decrease both the low-density lipoprotein cholesterol (LDL-C) and the serum cholesterol levels (Chen and Liu 2020).

In most human's diet, unsaturated fatty acids (UFAs) are found in a *cis* configuration. However, *trans* fatty acid (TFA) configurations were found in milk. The TFA has been linked to a negative effect on human health. It resulted from partial hydrogenation of UFAs (Amores and Virto 2019). Although conjugated linoleic acids (CLAs) have a TFA configuration resulting from partial biohydrogenation in the rumen, it has been separated from TFA (Chen and Liu 2020). Furthermore, CLAs may have different health benefits from TFAs, such as anti-cancer and anti-atherosclerosis activities (Despal et al., 2021a). Therefore, many strategies have been planned to increase the CLA content in milk (AbuGhazaleh, 2008; Oliveira et al., 2018; Prieto-Manrique et al., 2018; Pi et al., 2019). One of the strategies is supplementation with oil rich in UFA such as fish oil, sunflower rubber seed oil (AbuGhazaleh, 2008), flaxseed oil (Pi et al., 2019), safflower oil (Shi et al., 2015), palm oil prill fat (Riestanti et al., 2021), canola oil and soybean oil (Loor and Herbein, 2003).

Studies showed that addition of unprotected UFA to increase milk CLA content impaired rumen fermentation, feed and fiber digestibilities that induced milk fat depression (Hussein et al., 2013; Baldin et al., 2014; Pi et al., 2019). Unsaturated lipid supplements affect the hydrogenation process and result in different intermediate products in the rumen. Identifying precursors leading to the production of 18:1 and 18:2 isomers with a *trans*10 double bond in the

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rumen is of interest because they depress milk fat synthesis (Loor and Herbein, 2003). Canola oil contains about 12% α-linolenic acid (omega-3) and 65% oleic acid (Ghazani and Marangoni 2016). It contains high *cis*9-18:1, an omega-3. Soybean oil meal contained 10% palmitic acid (16:0), 4% stearic acid (18:0), 18% oleic acid (18:1), 55% linoleic acid (18:2), and 13% linolenic acid (18:3). It also contains high 18:2n-6. Supplementation of fat high in triglyceride-bound 18:2n-6 produced high *trans*11-18:1 and *cis*9, *trans*11-18:2 accumulations that could alter the profiles of intermediates product in the rumen, thereby affecting the amounts available for absorption in the small intestine. Palm oil contained less UFA than soybean and canola oils. It contained 50% SFA (80% palmitic (C16:0), 10% stearic (C18:0) and myristic (C14:0), 40% oleic (C18:1), and 10% polyunsaturated linoleic (C18:2) and linolenic acid (C18:3). However, this oil is more available and affordable to dairy farmers. This study aimed to determine the optimum canola, soybean, and palm oil supplementation level that does not impair the dairy ration's fermentation and digestibility.

MATERIALS AND METHODS

Ethical regulation

As a source of inoculant in the *in vitro* study, rumen liquor was collected from two fistulated Holstein Frisian bulls kept in the field of laboratory Dairy Nutrition, Department of Animal Nutrition and Feed Technology, Faculty of Animal Science, IPB University. Licensed veterinarians conducted the surgery for fistula implantation. The surgery- and animal handling and care followed the IPB University Animal Ethics Committee regulation.

Dairy cattle ration preparations

This experiment's basic dairy cattle ration includes 58.28% Napier grass, 33.62% concentrate, and 8.10% tofu waste. In addition, oils were added at 0% (B0), 1% (B1), 2% (B2), and 3% (B3) of canola, soybean, or palm oils, respectively. The composition of each ration and their nutrient content are shown in Table 1.

Table 1 - Ingredient and nutrient content of experimental rations

Ingredients and Nutrient contents	Rations			
	B0	B1	B2	B3
Ingredients				
Napier grass	58.28	57.7	57.14	56.58
Concentrate	33.62	33.29	33.96	32.64
Tofu waste	8.10	8.01	7.94	7.86
Oils (canola, soybean or palm)	0	1	2	3
Nutrient contents				
Ash	9.98	9.88	9.78	9.68
Crude protein	12.03	11.91	11.79	11.67
Ether extract	2.54	3.47	4.45	5.37
Crude fiber	22.47	22.25	22.02	21.81
Calcium	0.667	0.660	0.653	0.646
Phosphorus	0.481	0.476	0.471	0.467
TDN	57.17	58.42	59.67	60.80

TDN = total digestible nutrients, B0, B1, B2, and B3 = ration with addition 0, 1, 2, and 3% of oils.

Fermentability measurement

Two fistulated Holstein Frisian dairy bulls breed were used as an inoculant source. The rumen liquid was taken in the morning before feeding by filtering the content with two-fold cheese cloths. It was then kept in a warm container and transported into the laboratory. Feeds fermentability measurements were conducted following Tilley and Terry's first stage (Tilley and Terry, 1963). First, the 0.5 g sample was placed in a 100 ml fermentor tube, and 40 ml McDougall buffer and 10 ml rumen liquid were added. The tube was then aerated with CO_2 for 30 seconds to build an anaerobic condition, closed with ventilated rubber stopper, and placed in a 39°C water shaker bath. The fermentation lasted for 4 hours and was stopped by adding two drops of saturated $HgCl_2$. Afterwards, the tube was centrifuged at 3000 rpm for 15 minutes, and then the supernatant was collected and stored chills until observation of fermentability parameters. The fermentability parameters including pH, ammonia (NH3), and volatile fatty acid (VFA) concentrations were observed. The pH was measured using the Hanna HI98191 pH meter. Ammonia was measured using the Conway method, while VFA was measured using the steam distillation method.

Digestibility measurement

Digestibility measurement was conducted following a two-stage method (Tilley and Terry, 1963). The first is fermentative digestion, which followed a procedure similar to the fermentability measurement above but lasted 48 hours. After cancelling the fermentation activity, the tube was centrifuged at 3000 rpm for 15 minutes and the supernatant was removed. In the second stage, 50 ml 2% HCl-pepsin was added to the tube and incubated aerobically in a 39°C shaker water bath for 48 hours. Afterwards, the tube was filtered with a predetermined weight of Whatman paper no 41. The

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residue was dried at 60°C oven for 48 hours. Part of the residue was used for NDF and ADF analysis, and the rest was dried in a 105°C oven to determine dry matter (DM) residue. Incineration was then carried out in a 600°C oven for 6 hours to determine ash residue. NDF and ADF feed and residues were determined using fiber analyzer ANKOM 200. The measurement followed the AOCS standard procedure Ba 6a-05 (AOCS, 2005). The digestibility of DM, OM, NDF, and ADF was calculated by subtracting the residue from the sample and expressed as percentages.

Study design and data analysis

The experiment used a 3 x 4 factorial block design with four replications. Factor A is oil types consisting of A1 = canola oil, A2 = soybean oil, and A3 = palm oil. Factor B is oil supplementation levels consisting of B0 = 0%, B1 = 1%, B2 = 2% and B3 = 3%. The SPSS version 20 was adopted for data analysis using analysis of variance (ANOVA) followed by the Tukey test from SPSS version 20 (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Feed fermentability

Feed fermentability that measured from pH, ammonia (NH_3), and VFA parameters is shown in Table 2. The results showed that oil type did not significantly influence fermentability, but its level influenced the ammonia and VFA concentrations as well as the number of protozoa. Moreover, addition of oil significantly ($P<0.05$) reduced the ammonia concentration and protozoal number, but increased VFA concentration significantly ($P<0.05$). The pH value in ranges 6.25 to 7.3 was considered to be suitable for rumen microbial growth (Satter and Slyter, 1974). The normal pH value was found in this study due to the forage was used as the main composition in the ration. The addition of oils slightly reduced the pH from 7.02 to 6.7. Riestanti et al. (2020) reported consistent results, but Pi et al. (2019) showed higher results. The difference in the pH between the two studies is attributed to the ration and oils type used.

Table 2. Fermentability of feeds as the impact of different types and levels of oil supplementation

Items	Levels	Canola	Soybean	Palm	Average
pH	0	7.02±0.30	7.02±0.30	7.02±0.30	7.02±0.30
	1	6.66±0.27	6.69±0.26	6.60±0.28	6.65±0.05
	2	6.65±0.36	6.80±0.27	6.75±0.40	6.73±0.09
	3	6.70±0.27	6.80±0.27	6.74±0.25	6.75±0.06
	Average	6.76±0.18	6.83±0.14	6.78±0.18	
NH_3 (mM)	0	6.51±1.03	6.51±1.03	6.51±1.03	6.51±1.03^{a}
	1	3.75±0.34	3.75±1.03	4.32±0.53	3.94±0.33^{b}
	2	3.30±0.20	4.43±0.36	3.86±0.40	3.86±0.57^{b}
	3	3.53±0.86	4.66±1.61	3.97±0.52	4.05±0.57^{b}
	Average	4.28±1.51	4.84±1.19	4.67±1.25	
VFA (mM)	0	68.11±3.29	68.11±3.29	68.11±3.29	68.11±3.29^{b}
	1	105.1±28.6	92.16±6.47	140.3±70.5	112.5±24.9^{ab}
	2	145.6±35.1	130.7±22.0	152.9±52.8	143.0±11.3^{a}
	3	138.6±53.1	138.2±24.0	162.0±33.4	146.2±13.6^{a}
	Average	114.4±35.5	107.3±33.0	130.8±42.8	
Protozoa (log/ml)	0	6.63±0.10	6.63±0.10	6.63±0.10	6.63±0.10^{a}
	1	6.32±0.38	6.49±0.08	6.52±0.06	6.44±0.11^{b}
	2	6.37±0.02	6.38±0.16	6.47±0.14	6.40±0.06^{bc}
	3	6.25±0.02	6.26±0.10	6.19±0.05	6.23±0.05^{c}
	Average	6.40±0.17	6.44±0.16	6.46±0.19	

NH_3 = ammonia, VFA = volatile fatty acids, different superscript at different rows in the same parameter, expressed a significantly different ($P<0.01$) between the level of oils. The different superscripts at the different columns in the same parameters expressed a significantly different ($P<0.05$) between oil types.

The addition of oils at 1% reduced ammonia concentration to 4 mM. However, the concentration was still sufficient to support microbial growth (Satter and Slyter, 1974). The alteration of rumen fermentation after the addition of unprotected oils was reported by Pi et al. (2019). However, the ammonia concentration in their study was higher (8.99 - 9.10 mM after adding 4% rubber seed and flaxseed oils). The higher ammonia concentration reported by Pi et al. (2019) is due to the *in vivo* study used. Riestanti et al. (2021) reported ammonia concentration in the range of 8 - 10 mM in an *in vitro* study conducted after adding 2 - 6% protected oil. Furthermore, Jayanegara et al. (2021) reported an increasing ammonia concentration after adding 1 - 5% maggot oil, which contained short-medium fatty acids (SMFA). The short-chain fatty acid (SCFA) supplementation effect on rumen fermentation is not well documented. In contrast, medium-chain fatty acids (MCFA) were shown to disrupt rumen metabolism by decreasing the number of protozoa, depressing fiber

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degradability (Dohme et al., 2004). Also, 4% MCFA supplementation level has been reported to disrupt microbial populations (Hristov et al., 2009).

Here, oils addition in a concentration of up to 3% could increase the VFA concentration up to 146 mM which is similar to the *in vitro* study of Riestanti et al. (2021). In contrast, Jayanegara et al. (2021) showed no influence of maggot oil supplementation up to 5% on VFA concentration. In the *in vivo* study of Pi et al. (2019), it was demonstrated that ammonia and VFA decreased with oil supplementation. The addition of fat in dairy ration to increase the energy content in high producing cattle is commonly conducted to prevent high concentrate usage and milk fat depression (Hristov et al., 2009). However, the result varies depending on the saturation of FAs (Avila et al., 2000), fat level, and the diet (Palmquist and Jenkins, 1980).

Protozoal found in this study was 6.23 - 6.63 log/m, and the oil supplementation reduced this number significantly at $p<0.05$ but still in the normal range of the rumen protozoal population (McDonald et al., 2010). This result is similar to Riestanti et al. (2021) and Jayanegara et al. (2021) who reported a slight increase in the protozoal number as maggot oil level increased. Furthermore, Jayanegara et al. (2021) reported that C12:0 fatty acid (rich in maggot oil) was toxic for methanogens, partially eliminating ciliate protozoa, and depressed fiber fermentation by cellulolytic microbes (Machmüller et al., 2002).

Feed digestibility

The digestibility of feed and fiber is shown in Table 3. The DMD and OMD ration found in this experiment was more than 50%, except for the high level of Canola oil supplementation that produced below 50%. However, the DMD and OMD here were lower than the normal ration digestibility for dairy cattle (67 - 71%) (Zahera et al., 2015; Hasanah et al., 2017) and dairy goat's ration (60 - 65%) (Despal et al., 2017). Moreover, low DMD and OMD were due to the addition of oil (Riestanti et al., 2021) and the *in vitro* method used in the assessment. According to the *in vivo* conditions, lower digestibility means higher nutrient excess to the environment.

Table 3 - Digestibility of feeds as an impact of different types and levels of oil supplementation

Items	Level	Canola	Soybean	Palm	Average
DMD	0	63.55±4.13	63.55±4.13	63.55±4.13	63.55±0.00^{a}
	1	59.30±1.33	61.08±3.67	54.0 6±11.44	58.15±3.65^{ab}
	2	54.05±6.90	60.57±4.12	56.12±4.64	56.91±3.34^{ab}
	3	48.67±12.1	53.33±2.06	60.62±3.36	54.21±6.03^{b}
	Average	56.40±6.46	59.64±4.40	58.59±4.30	
OMD	0	62.32±2.49	62.32±2.49	62.32±2.49	62.32±0.00^{a}
	1	58.07±0.88	59.76±1.84	60.16±5.98	59.33±1.12^{ab}
	2	51.85±7.77	56.34±2.26	57.29±6.94	55.16±2.91^{bc}
	3	45.29±12.0	50.76±3.27	56.01±3.27	50.69±5.37^{c}
	Average	54.39±7.44	57.3±5	58.95±2.85	
NDFD	0	64.41±6.44	64.41±6.44	64.41±6.44	64.41±6.44
	1	52.50±13.7	58.53±4.46	59.35±5.66	56.79±3.75
	2	58.87±7.16	56.75±8.22	60.00±5.49	58.54±1.66
	3	55.38±6.34	60.31±5.63	57.02±8.70	57.57±2.51
	Average	57.79±5.13	60.00±3.28	60.20±3.09	
ADFD	0	50.70±11.4	50.70±11.4	50.70±11.4	50.70±11.4
	1	37.41±19.1	45.88±8.58	43.06±12.2	42.12±4.31
	2	46.66±16.9	42.73±13.8	43.94±11.6	44.45±2.02
	3	40.01±11.8	45.64±11.8	42.86±14.6	42.84±2.82
	Average	43.70±6.09	46.24±3.31	45.14±3.74	

DMD = dry matter digestibility, OMD = organic matter digestibility, NDFD = neutral detergent fiber digestibility, ADFD = acid detergent fiber digestibility. Different superscripts at different rows in the same parameter expressed a significantly different ($P<0.01$) between the level of oils. The different superscripts at the different columns in the same parameters expressed a significantly different ($P<0.05$) between oil types.

Oil types have no significant effect on digestibility, but their levels affect DMD and OMD. However, fiber digestibility was not significantly reduced. Furthermore, addition of 1% and 2% oils did not significantly reduce OMD and DMD, respectively. The insignificant difference in fiber digestibility is due to the high variation of data shown by the high standard deviations.

The negative effect of fat or oil supplementation on rumen fermentation does not apply to all conditions (Palmquist and Jenkins, 1980). Several reported studies showed decreasing in digestibility coefficient due to fat supplementation (Pi et al., 2019; Riestanti et al., 2020). It was reported that oil supplementation specifically reduced the fiber digestion due to depression in the cellulolytic bacterial population (Riestanti et al., 2021). The addition of oils set in this experiment comprised less than 5% of the total ether extract in the diet. However, the unprotected type of the used oil might impair the rumen fermentation.

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Furthermore, the oil's energy supplemented did not compensate for the declination of digestibility. It might be caused by the *in vitro* method that did not measure post ruminal digestibility (Tilley and Terry, 1963). Many studies also compared the different effects of the *in vitro* and *in vivo* oil and fat supplementation on digestibility (Riestanti et al., 2020; Riestanti et al., 2021). In the *in vivo* study, the dependency of ruminants on non-glucose metabolites for energy metabolism explains these results. In lactating cattle, daily FA output in the milk might exceed daily intake (Palmquist and Jenkins, 1980). Therefore, lipid metabolism plays an essential role in these different results. However, it is not easy to be imitated in an *in vitro* study.

CONCLUSION

The *in vitro* addition of unprotected canola, soybean, or palm oil as sources of UFAs to increase CLA content in milk did not influence rumen fermentability and feed digestibility. Oil levels could influence fermentability and digestibility. The addition of up to 1% of all of type oils did not impair feed digestibility. Therefore, it is recommended to protect the oil from biohydrogenation in the rumen to increase its utilization in the dairy ration. The use of *in vivo* study is also recommended.

DECLARATIONS

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Authors' contribution

Despal D. designed the study, searched for funding, supervised the laboratory work, wrote and reviewed the manuscript. Permana I.G. and Zahera R. designed the study, searched for funding, supervised the laboratory work, and reviewed the manuscript. Irmadani D. designed the study, conducted the laboratory work, and wrote the manuscript. Nuraina N. supervised the laboratory work, analyzed the data, edited and reviews the manuscript.

Conflict of interests

The authors have not declared any conflict of interest.

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MOLYBDENUM SUPPLEMENTATION OF FAT-TAILED EWES DIETS IN AN ARID REGION

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Supporting Information

ABSTRACT: The study aimed to establish the normal molybdenum requirements for open ewes of meat-and-fat breeds in arid climatic conditions. The study was carried out in the production conditions of the Buddha farm of the Republic of Kalmykia (Russia). The norm has been established based on a detailed study of the molybdenum content in organs and tissues and the degree of its absorption from the diet, considering the endogenous losses. It has been established that the norm of molybdenum is 4.5 mg per head per day, 2.8 mg per 1 kg of dry matter of the diet and 0.80 mg per 1 kg of live weight of a single ewe. Recommendations have been made to solve the problem of molybdenum deficiency in the diet of open ewes of meat-and-fat breeds in arid climatic conditions of Republic of Kalmykia.

Key Words: Open ewes, Norm, Element, Concentration, Kalmyk breed

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INTRODUCTION

Among the factors determining the usefulness of feeding ewes, microelements, including molybdenum, occupy a significant place. The significance of molybdenum can be judged by the fact that it is an integral part of such enzymes as xanthine oxidase, aldehyde oxidase, and sulfytoxidase (Kalnitskii, 1978; Novotny, 2011; Novotny and Peterson, 2018). This trace element is essential for plants, animals and micro-organisms especially those involved in the bacterial digestibility of fiber in the ruminant pancreas (Ellis et al., 1958; Kalnitskii, 1978; Novotny and Peterson, 2018). Molybdenum, like other elements, takes an active part in the metabolism in the body of animals and thereby has a significant impact on their productivity (Moskalev 1985; Ellis et al., 1958).

On the other hand, high intake of molybdenum into the body of animals, especially cattle and sheep, leads to metabolic disorders, severe toxicosis, accompanied by diarrhea, anemia, paleness of the hair, and a decrease in body weight (Merkur'eva 1970 and Russell et al., 2001), because induce secondary copper deficiency in these animals (Novotny and Peterson, 2018, Stepanova et al., 2020). A very high intake of molybdenum in rodents also led to kidney and liver histological damages, reproductive failure, renal failure, anemia, bone deformities and even growth retardation (Russell et al., 2001; Novotny, 2011).

Therefore, it is very important to control the intake of this element into the body of animals, considering its content in the diet feed. A review of the literature shows that the issues of molybdenum content in the diet have been studied in more detail on the example of dairy cows and sheep of the meat-and-wool and wool-and-meat breeds and molybdenum still has not been included in the mandatory controlled indicators that should be taken into account when compiling diets. As for the open ewes of the meat-and-fat breeds in the conditions of the arid zone of their breeding, there is currently no information regarding their requirement for this element. Considering these circumstances, the purpose of this study was to investigate the molybdenum metabolism in open ewes, and developing standards for the requirements of this element for the Kalmyk fat-tailed breed in the conditions of the arid zone.

This work was performed to A) determine the molybdenum content in the organs, tissues, and contents of the gastrointestinal tract of ewes during the period of their being in an open state; B) determine the degree of absorption of this element from the diet; and C) calculate the daily intake requirement and establish the norm of molybdenum for fat-tailed open ewes by the factorial method.

MATERIALS AND METHODS

This study was carried out in the production conditions of the Buddha farm at the Republic of Kalmykia (Russia) on ewes of the Kalmyk fat-tailed breed after the weaning of the lambs and before mating, 3 heads of each period with a live

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weight of 55-57 kg. During the experiment, the animals were kept in individual cages, feeding was carried out according to the recommended standards of the Russian Academy of Agricultural Sciences (RASKhN) (Kalashnikov et al. 2003), considering the chemical composition of local feeds.

The composition of the diet of open ewes included grass of herb-bunchgrass pasture (3.5 kg), alfalfa hay (0.3 kg), barley (0.1 kg), table salt (11 g), copper sulfate (29 mg), zinc sulfate (124 mg), manganese sulfate (58 mg) and cobalt chloride (1.8 mg). The feed contained 3.193 mg of molybdenum.

To study the molybdenum content in organs, tissues, and in the whole body, on the day of the end of each balance experiment, 3 heads of sheep were slaughtered after the weaning of the lambs and before mating. At that time, the mass of organs, tissues, and contents of the digestive tract of sheep was determined. In those samples, the concentration of molybdenum was determined on an atomic absorption spectrometer.

The factorial method was used to calculate the requirement of molybdenum in open ewes:

1. Based on the data on the concentration of molybdenum in organs and tissues and the contents of the digestive tract, the total content of this element in the body was determined;
2. The amount of molybdenum that is deposited during the idle period of ewes and per day was determined;
3. Endogenous molybdenum losses were determined with feces according to Bell et al. (1964); Udris and Neiland (1976), and the losses with urine were calculated directly;
4. The daily deposition of molybdenum in the body of ewes and endogenous losses with feces and urine were summed up and based on this, the true daily total requirement for this element was established;
5. According to the results of balance experiments, considering endogenous losses in feces, the true digestibility of molybdenum from diets was revealed as a percentage using the following formula:

$$D = \frac{I-(E-En)}{I} \times 100$$

Where: D is the true digestibility of the element (%); I is the intake of the element with the diet (mg); E is the excretion of an element with feces (mg); En is the endogenous excretion of an element with feces (mg);

6. The established total true requirement was divided by the percentage of true absorption and, as a result, the recommended amount of the element that should be contained in the diet was obtained.

Statistical analysis

The digital material of the experiments was processed biometrically according to E.K. Merkureva's method (Merkureva 1970) on a computer using the program "Statistics" version -2.6. The results obtained were studied and compared by the group method. The difference in mean scores between groups was considered significant at the level of probability (P=0.05) determined by Student's t-test.

Ethical approval

The work was carried out in accordance with the passport of scientific directions of the Department of Animal Science named after Professor S.A. Lapshin National Research Mordovian State University named after N.P. Ogaryov on the topic "Optimization of feeding of highly productive animals and poultry using digital technologies in the development of resource-saving technologies for the production of livestock and poultry products", Ogaryova, protocol No. 5 of 08/20/2018).

RESULTS AND DISCUSSION

The results of the study showed that the Kalmyk fat-tailed open ewes fed dietary molybdenum of 2.86-2.82 mg, daily (Table 1). Out of this amount of the element, 60.14% or 1.72 mg per day was absorbed by open ewes after the weaning of the lambs (Table 1). During the idle period, the absolute value of molybdenum absorption decreased by 0.08 mg, and the relative value decreased by 1.99%. Molybdenum excretion in fat-tailed open ewes mainly occurred through the gastrointestinal tract and in small amounts (0.24-0.22 mg/day) through the kidneys.

The main mass of endogenous molybdenum was excreted from the body in the urine by open ewes of the Kalmyk fat-tailed breed. It should be noted that the total loss of this element during the idle period of fat-tailed ewes did not change significantly and remained at the level of 1.50-1.52 mg, and the deposition in the body decreased slightly from 1.36 to 1.30 mg. In our studies, it was found that the idle period of ewes had a significant effect on the distribution and concentration of molybdenum in their tissues and organs (Table 2). Thus, the concentration of this element in the blood of ewes after the weaning of the lambs was 1.68 mg/kg, and by the mating period, it increased by 2 times reaching up to 3.40 mg/kg ($P<0.001$). Due to an increase in the concentration of molybdenum, its total amount in the blood also increased by 2.3 times or from 5.77 mg to 13.20 mg ($P<0.05$).

Of all the tissues of open ewes, bone tissue (81.81-81.769 mg/kg) and skin with wool (15.194-25.721 mg/kg of raw tissue) had the highest concentration of molybdenum ($P<0.05$). At the same time, it should be noted that if the concentration of molybdenum during the idle period in the bone tissue remains at the same level (Bell et al., 1964), then in the skin with wool, it increases by 1.7 times ($P<0.001$) as it follows from the current study (Table 2). Then the tissues can be arranged in descending order by molybdenum content in the following way: nerve tissue, muscle tissue, peri-renal adipose tissue, internal adipose tissue. The smallest amount of molybdenum (0.145-0.100 mg/kg) is contained in the fat

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tail. By the mating period of fat-tailed ewes the concentration of the element in the tongue increased by 19.8% (P>0.05), by 2.5 times in the udder (P>0.001) and in the uterus, on the contrary, it decreased by 5.7% (P>0.05).

The total molybdenum content in the muscle tissue increased by 1.4 times by the mating period of ewes (P<0.001), while in the bone tissue it increases by 6.2% (P<0.001), in the skin with wool by 1.7 times (P<0.001), in the internal fat by 2 times (P<0.05), in the perirenal fat by 1.6 times (P>0.05), and in the brain by 1.2 times (P<0.05). At the same time, it should be noted that during the idle period, the absolute molybdenum content in the fat and udder did not change significantly, in the tongue it decreased by 26.6% (P>0.05), and in the uterus, on the contrary, it increased by 2.4% (P>0.05).

Conducted studies have also shown that molybdenum is unevenly distributed in the internal organs of open ewes of the meat-and-fat breed. It was found that out of the internal organs the highest concentration of this element was contained in the liver. The liver contains from 25.128 to 28.853 mg of the element/kg of raw tissue, which indicates the active participation of molybdenum in the metabolic processes of the body of open ewes. At the same time, the total amount of molybdenum in this organ also increases.

Of the other internal organs, the spleen also has a high concentration of this element (10.678-43.460 mg/kg). The concentration of molybdenum in it is 3.9 and 17.3 times higher than in the heart, 6.2 and 25.4 times higher than in the lungs, and 10 and 32.5 times higher than in the kidneys. As for the total amount of the element, the internal organs can be arranged in descending order as follows: liver (20.00-22.50 mg), spleen (0.903-4.204 mg), lungs (0.885-0.973 mg), heart (0.619-0.624 mg), and kidneys (0.102-0.135 mg). The absolute amount of the element in the heart during the idle period of the ewes does not change significantly. In the lungs, it increases by 9.9%. In the liver, the dynamics of this indicator is also 12.5% mg. The absolute amount of molybdenum in the kidneys of ewes during the study period increased by 32.3%, and in the spleen by 4.6 times (Table 2). Our studies have also shown that the amount of this element in the walls of the digestive tract of meat-and-fat open ewes is also subject to fluctuations. Of all the departments of the stomach, the manifold walls had a higher concentration of molybdenum (25.494-37.760 mg/kg), and the concentration in the abomasum walls was low (1.547-1.910 mg/kg). It should be noted that during the idle period, the concentration of the element in the manifold increases by 1.5 times (p <0.05), and in abomasum by 1.2 times (P<0.05). The remaining sections of the gastrointestinal tract of open ewes can be arranged in the descending order by molybdenum content: large intestine (13.811-17.676 mg/kg), rumen (5.499-6.692 mg/kg), small intestine (2.857-6.393 mg/kg), honeycomb stomach (2.016-3.051 mg/kg) and abomasum (1.547-1.910 mg/kg).

In all parts of the gastrointestinal tract of ewes, the absolute amount of molybdenum increased during the idle period, and its maximum content was observed in the large intestine (16.015 - 18.550 mg) and the walls of the rumen (5.596-8.130 mg). Low content of this element was observed in the honeycomb stomach (0.312-0.433 mg) and abomasum (0.729-0.767 mg) (P>0.05). Our studies also showed that the accumulation of molybdenum in the contents of the digestive tract of fat-tailed open ewes has differences. Thus, the concentration of this element during the studied period increases by 1.5 times in the chyme of the rumen (P<0.05), by 5.4% in the chyme of the honeycomb stomach (P>0.05), by 10.8% in the chyme of the manifold (P<0.05), by 5.4% in the chyme of the honeycomb stomach (P>0.05), by 22.7% in the chyme of the abomasum (P<0.05), by 5.4% in the chyme of the honeycomb stomach (P<0.05), by 2.3 times in the chyme of the small intestine (P<0.001) and by 2.8 times in the chyme of the large intestine (P<0.01).

Of the total absolute amount of molybdenum contained in the chyme, the main share (74.90-77.90%) falls on the rumen, 10.2-13.0% on the chyme of the large intestine, and 8.4-10.0% on the chyme of the small intestine. The absolute molybdenum content in the body of ewes after the weaning of the lambs was 805.12 mg, and by the mating period, it was increased by 25.4%, and reached up to 1010.32 mg.

As a result of our study, it was found that the absolute deposition of molybdenum in the body of ewes during the idle period increased from 805.12 to 1010.32 mg or by 1.25 times, i.e. during this period, 205.12 mg of molybdenum was deposited in their body. At the same time, the daily deposition of the element equals 2.28 mg (Table 3). Calculations also showed that the true molybdenum requirement for open ewes of the meat-and-fat breed with a live weight of 55-57 kg equaled 2.62 mg per 1 head per day to ensure their normal functioning and obtain high gains (Table 3).

Table 1 - The absorption of molybdenum from the diet by open ewes, mg

Indicators	Ewes after the weaning of the lambs	Ewes before mating
Taken with food and water, mg	2.86±0.02	2.82±0.01
Excreted with feces total, mg	1.26±0.03	1.30±0.02
incl. endogenous losses, mg	0.12±0.01	0.12±0.01
Visible absorption, mg	1.60±0.02	1.52±0.01
True absorption, mg	1.72±0.01	1.64±0.01
True absorption, %	60.14±0.53	58.15±0.07
Excreted in urine, mg	0.24±0.01	0.22±0.01
Total excreted, mg	1.50±0.02	1.52±0.01
Retained in the body, mg	1.36±0.01	1.30±0.01
Percentage of the amount taken	47.55±0.50	46.10±0.11

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Table 2 - The molybdenum content in the tissues and organs of open ewes, mg

Indicators	Concentration, mg/kg		The total amount, mg	
	Idle periods		Idle periods	
	Ewes after the weaning of the lambs	Ewes before mating	Ewes after the weaning of the lambs	Ewes before mating
Blood	1.68±0.19	3.40±0.14*	5.77±0.29	13.20±0.07***
Muscle tissue	4.83±0.06	6.305±0.12	80.10±0.67	116.55±0.04***
Bone tissue	81.81±3.34	81.769±2.89	529.80±2.12	563.00±2.51
Leather with the wool coat	15.194±0.22	25.721±0.44***	94.13±0.94	162.00±1.73***
Internal fat	0.428±0.01	0.654±0.03	0.218±0.03	0.443±0.02*
Pararenal fat	1.512±0.12	0.928±0.01	0.119±0.02	0.190±0.03
Fat tail	0.145±0.03	0.100±0.05	0.55±0.03	0.530±0.01
Brain	5.960±0.20	6.923±0.07	0.803±0.03	0.955±0.03*
Tongue	3.398±0.37	4.070±0.06*	0.365±0.02	0.268±0.03
Heart	2.738±0.25	2.504±0.10	0.619±0.06	0.624±0.03
Lungs	1.704±0.04	1.708±0.03	0.885±0.05	0.973±0.02
Liver	25.128±1.75	28.853±0.42	20.00±0.80	22.50±0.16
Kidneys	1.065±0.02	1.338±0.09	0.102±0.02	0.135±0.02
Spleen	10.678±0.51	43.460±1.66	0.903±0.02	4.204±0.08
Uterus	5.142±0.17	4.854±0.13	0.592±0.02	0.606±0.03
Udder	1.185±0.08	3.00±0.19***	0.258±0.03	0.268±0.03
Rumen	5.499±0.18	6.692±0.04	5.596±0.08	8.130±0.03
Honeycomb stomach	2.016±0.06	3.051±0.07	0.312±0.03	0.433±0.03
Manifold	25.494±1.06	37.760±1.30*[b]	4.318±0.02	7.160±0.03
Abomasum	1.547±0.07	1.910±0.02*[a]	0.755±0.03	0.907±0.03
Small intestine	2.857±0.11	6.393±0.09	3.702±0.04	7.990±0.04
Large intestine	13.811±0.18	17.676±0.32	16.015±0.01	18.550±0.07
Contents of the rumen	5.277±0.14	10.323±1.14*	30.560±0.03	60.42±0.06
Contents of the honeycomb stomach	0.764±0.04	0.807±0.02	0.277±0.03	0.266±0.03
Contents of the manifold	3.956±0.38	4.383±0.10	0.776±0.03	0.950±0.04
Contents of the abomasum	0.811±0.03	0.995±0.04	0.275±0.02	0.313±0.05
Contents of the small intestine	4.083±0.08	9.567±0.12***	3.305±0.04	8.13±0.03
Contents of the large intestine	7.589±0.23	21.310±1.68**	4.015±0.05	10.52±0.04
Total			805.12	1,010.32

* P<0.05; **P<0.01 ;*** P<0.001; [a] the lowest result; [b] the highest.

Table 3 - The daily molybdenum requirement for open ewes and the molybdenum norm in diets, (mg)

Indicators		Ewes after the weaning of the lambs	Ewes before mating
Total molybdenum content in the body		805.12	1010.32
Total deposition of molybdenum in the body during the period		-	205.12
Daily deposition of molybdenum		-	2.28
Endogenous losses	with feces	0.12	0.12
	with urine	0.24	0.22
	total	0.36	0.34
True daily molybdenum requirement		-	2.62
True digestibility from the diet, %		-	58.15
The actual daily norm in the diet	per 1 head	-	4.50
	per 1 kg of dry matter of the diet	-	2.80
	per 1 kg of live weight	-	0.80

The studies on the ewes of the Kalmyk fat-tailed breed in the arid zone conditions were carried out for the first time. The limited studies have been conducted on effect of dietary molybdenum in sheep (Bampidis et al., 2019); therefore, the information about the rationing of molybdenum for ewes of the meat-fat direction of productivity was not found among the scientific studies published to the moment. It makes it impossible to give a comparative assessment of the obtained results.

CONCLUSION

Thus, based on the data on the molybdenum concentration in the organs and tissues of open ewes of meat-and-fat breeds, it can be concluded that the content of this element increases both, the molybdenum concentration in the organs

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and tissues, due to an increase in the mass of organs and tissues, and an increase in the concentration of elements in them, and is also determined by the duration of the idle period of ewes. Based on data on the molybdenum daily deposition in the body, its content in organs and tissues, the degree of absorption from the diet, and endogenous losses with feces and urine, we have calculated the molybdenum requirement for open ewes of the meat-and-fat breed in arid climatic conditions. Since only 58.6% of this element is absorbed from the diets, open ewes should receive 4.50 mg of molybdenum per 1 head per day in the daily diet. There is 2.80 mg per 1 kg of dry matter of the diet, and 0.80 mg of molybdenum per 1 kg of live weight.

DECLARATIONS

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Author's contribution

D. Sh. Gayirbegov performed conceptualization, methodology, formal analysis, validation, writing original, review, statistics and editing; D. B. Mandzhiev performed methodology, review, editing, and validation; and T. B. Tyurbeev performed conceptualization, review and editing.

Competing interests

The authors declare that they have no competing interests.

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TRADITIONAL MANAGEMENT PRACTICES AND PRODUCTION POTENTIAL OF BEEKEEPING IN ERER ZONE OF SOMALI REGIONAL STATE, ETHIOPIA

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ABSTRACT: The study was carried out in Erer zone, Somali Region of Ethiopia, to assess beekeeping practices and production. A total of 156 respondents were selected randomly from two purposively selected localities based on beekeeping potential and interviewed using semi-structured questionnaire. The study revealed the majority of the respondents (85.9%) practiced traditional beekeeping system and hung their hives on trees away from the homestead in dense forest. The honeybee flora of the area consists mostly of nectar and pollen-producing trees and shrubs including *Grewia tenax*, *Grewia penicillata*, *Acaciamellifera*, *Acaciareficiens*, *Acaciatortilis*, *Acaciasenegal*, and *Acaciahorrida*. The average honey yield from traditional beehives was 4.85 kg, which is less than the national average yield (5 kg). The average honey yield from modern beehives, on the other hand, was 7.29 kg which is lower the amount obtained from other parts of the country. The major constraints of beekeeping are the high cost of modern hives and accessories, pests and predators of honey bees, water scarcity, shortage of bee forage, bee absconding and marketing. Birds, ants, spiders, wax moth, mice, lizards, and honey badgers were identified as the major honeybee pests and predators based on beekeeper responses. Pests and predators (37.8%), destroying nests during honey harvesting (26.9%), water scarcity (21.2%), and shortage of bee forage (14.1%) were the most common reasons for honeybee absconding. Beekeeping production remains low due to these constraints and traditional practices in the area. However, there is enormous potential and opportunity to increase honeybee production in the area. To improve the quantity and quality of honey yield in the area, significant extension and technical intervention, use of locally available beekeeping technologies, appropriate measures to manage honeybee pests and absconding, and training to enrich beekeepers' knowledge are necessary to be implemented.

Keywords: Bee forage, Beekeeper, Beekeeping practice, Hive, Honey production.

INTRODUCTION

Beekeeping is an important component of agriculture and rural development program of many countries. It helps to provide security in nutrition, economy and ecology (Jeil et al., 2020; Güler, 2021). Besides, it does not compete with other resources in the farming system, it is income generation activity and supplement annual income for the beekeepers through sell of bee products (honey, beeswax, and bee colonies). It also serves as a healthy food for consumers (FAO, 2015).

Ethiopia is the home of diverse fauna due to its varied ecological and climatic conditions (Beyene et al., 2016). This is the prime reason for the availability of large colony numbers in the country. In Ethiopia, three types of beehives (traditional, intermediate, and improved) are known, with more than 10 million colonies, from which more than 90% are traditional hives (CSA, 2021). Ethiopia is endowed with diverse agro-climatic zones, which are suitable for honey production.

The total annual honey production in the country is estimated about 129 million kilograms of which the greater portion is harvested from traditional beehives (CSA, 2021). Thus, while the country is a principal producer of honey, it has the potential to improve yields and harvest more if existing beekeepers are able to overcome significant issues regarding inputs, technical skills, and climate change adaptation (Dong et al., 2016; Gratzer et al., 2021). In order to exploit the country's production potential, the government has given consideration to developing the beekeeping subsector as a strategy for the reduction of poverty and the diversification of export commodities (Shapiro et al., 2015).

Attention is also given to upgrading the knowledge and skill of developmental agents and beekeepers so that they can develop better apicultural knowledge and skills that enable them to improve traditional beekeeping and increase the production of hive products (Girma et al., 2008). Furthermore, various non-governmental organizations intervene to assist

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the poor and the formation of beekeepers' cooperatives and unions in order to bring about significant changes in the increased supply and consistent quality of honey and beeswax, allowing smallholders and the country to benefit from the sub-sector (Reda et al., 2018). Besides, the federal and regional agriculture and livestock bureaus have improvement strategies aimed at increasing the quality and quantity of hive products (MoA and ILRI, 2013).

Despite the long tradition of beekeeping in Ethiopia, having the highest bee density and being the leading honey producer as well as one of the largest beeswax exporting countries in Africa, the contribution of this sector is very low. In Somali Region, large proportion of inaccessible lands for agriculture are covered with various types of trees, shrubs, bushes, and field flowers that make this part of the regions still to be potential for beekeeping. However, these resources are not being utilized and efforts must be made to address some of the major problems of beekeeping and to keep it productive in the sustainable manner. Erer zone is one of the potential areas in beekeeping and supplies the most beehive products in the region, such as honey and beeswax. However, there is no compiled and reliable information on beekeeping practices and its production potential. Therefore, this study was carried out to assess beekeeping practices and identify challenges and opportunities of honeybee production.

MATERIALS AND METHODS

Description of the study area

This study was conducted in Lagahida and Fiq districts of Erer zone of Somali Regional State, Ethiopia. Fiq district is bordered on the south by Hamero, on the western by Qubi, on the west by Mayamuluka, on the north by the Jigjiga Zone, on the east by the Jarar Zone, and on the southeast by Sagag. The elevation of this woreda is 1035 m. It has a weather of 32 ºC and 27% humidity. It has a latitude and longitude of 8°8'16" N and 42°17'36" E. Lagahida district is bordered on the south by Salahad, on the west by the Oromia region, on the north by Mayumuluka, and on the east by the Erer which separates it from Hamero.

Sampling technique and sample size

Multistage sampling technique was applied in this study. At the first stage, two districts namely Fiq and Lagahida were purposefully selected based on their beekeeping potential in consultation with pastoral development focal of the zone. At the second stage, a total of six kebeles (three from each district) were selected based on beekeeping potential. Lastly, a total of 156 beekeeping households (2 districts *3 kebeles *26 households) were selected for this study based on their willingness to participate.

Data collection and analysis

Semi-structured questionnaires, field observations, interview with key informants and focus group discussions were applied to collect the required data for this study. The data were analyzed using SPPS (Version 26.0) and were presented in the form of tables and figures.

RESULTS AND DISCUSSION

Household characteristics

Table 1 shows the household characteristics of the respondents. Majority of the respondents (96.8%) were men, with the remaining (3.2%) being women. This indicated that higher proportion of beekeeping activities are left for male, with no female participation. The age characteristics indicated that most of the respondents fell within the range of 31-45 years (55.2%), followed by 28.6% (46-55 years), 23.7% (<30 years) and only (14.3%) of the respondents were aged above 55 years. Peoples in the aforementioned age do have the ability, skill and strength to climb large trees and uplift the hive to hang on branches of large trees. This result demonstrates people in the most productive age engage in the beekeeping activity. The survey also revealed that the vast majority of respondents (96.8%) were married. Marriage fosters synergy within a farm family and serves as a custom for dealing with life's challenges in the rural community.

Out of the total number of beekeepers interviewed, approximately 80.8% were illiterate, while the remaining (19.2%) could read and write. Traditional practices predominate in the study area because the majority of respondents hardily understand and accept new technologies. The educational level of farming households is critical for understanding extension packages and the adoption of improved technologies, which in turn determines the community's development. For more advanced beekeeping, a good understanding of bee biology and behavior is necessary for better colony management. In general, education is an important tool that determines the level of transformation of knowledge to improve beekeeping practice.

Beekeeping practices

Type of beekeeping

Table 2 shows the types of beekeeping practiced by respondents in the study area. According to the investigation, three distinct types of beekeeping were used by the sample respondents in the study area based on their level of technological advancement. As a result, a large proportion of respondents (85.9%) practiced only traditional beekeeping,

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and the remaining (14.1%) practiced both traditional and movable frame hive beekeeping concurrently. It has also been observed that beekeepers constructed traditional hives using locally available materials and indigenous knowledge. The current study supported Getachew's (2018) report, which stated that a large proportion of sampled households in the Gesha district of Keffa zone practiced traditional beekeeping. The findings of the investigation are also consistent with other findings done in the Central, Northern, and South Western parts of Ethiopia, where traditional beekeeping systems predominate in rural areas (Getachew, 2018; Reda et al., 2018; Gratzer et al., 2021).

Traditional beehives construction

Traditional beehives were made of wood trees and hung from forest trees. Xagar (*Commiphora gileadensis*), Xoday (*Commiphora hodai Sprague*), and Barde (*Ficus glumosa del.*) were the most commonly available trees made from traditional hives in the study area.

Source of honeybee colony

The respondents in the study area own bee colonies from different sources. As indicated in Figure 1, the majority (84%) of the respondents obtained bee colonies by catching swarms whereas 8.3% and 7.7% obtained their colony from parents as a gift and by buying, respectively. This finding is in agreement with the report of Weldearegay and Anja (2017) who reported that majority (72%) of the respondents in Sude Woreda of Arsi Zone Oromia obtained bee colonies by catching swarms whereas 21 and 7% obtained their colony by buying them and from parents as gift, respectively.

Table 1 – Characteristics of the sampled households (%)

Variables		Fiq (n=78)	Lagahida (n-78)	Overall (n=156)
Sex	Male	97.4	96.1	96.8
	Female	2.6	3.9	3.2
Age (years)	<30	25.6	21.8	23.7
	31-45	51.3	58.9	55.2
	46-55	20.5	15.4	17.9
	>55	2.6	3.9	3.2
Marital status	Married	96.1	97.4	96.8
	Divorced	-	1.3	0.6
	Widowed	1.3	-	0.6
	Single	2.6	1.3	2
Educational status	Illiterate	76.9	84.6	80.8
	Read and write	23.1	15.4	19.2

Table 2 –Type of beehive (%) in the study area

Type of beehive	Fiq (n=78)	Lagahida (n-78)	Overall (n=156)
Traditional beehive	82.1	89.7	85.9
Traditional and modern	17.9	10.3	14.1

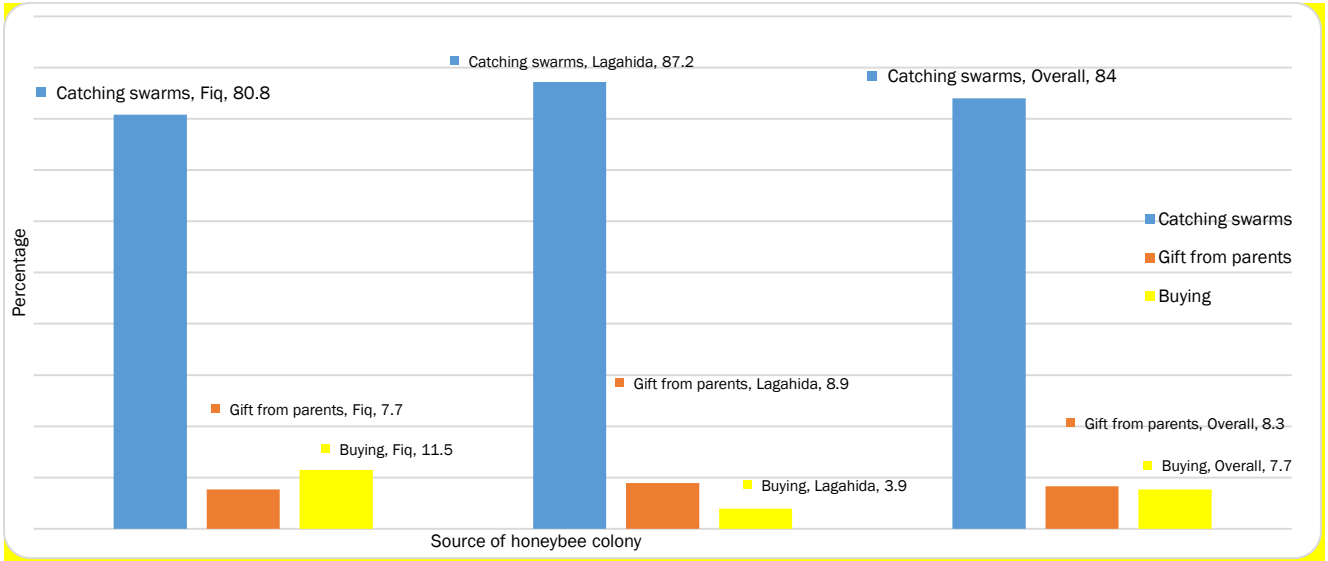

Figure 1 - Source of honeybee colony in the study area

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Placement of the beehive

The placement of beehive of sample respondents in the study area is presented in Table 3. According to the study, majority of beekeepers (73.1%) in the study area hang their hives on trees away from the homestead in dense forest. Considerable portion of the respondents (17.3%) kept their beehive in the backyard. Only a small percentage of respondents (9.6%) kept their hive in the backyard as well as hang on trees. The predominant honey production system in the study area is based on traditional beekeeping techniques dominated by forest and backyard beekeeping. Beekeepers in the study area prefer to hang their beehives in dense forest far away from residential areas, where there is plenty of bee forage and bee swarms are plentiful. The current findings are consistent with those of Fikru et al. (2015), who discovered that beekeepers in Jigjiga zone hang their beehives on trees and used a traditional beekeeping system.

The result of the current study is substantiated by Getachew (2018) who reported that about 55% of beekeepers in Gesha district of Southwest Ethiopia placed their beehives on branches of tree in the dense forest far away from their residential areas whereas the 23.1% of the respondents' hang on trees near homestead and about 1.9% of beekeepers kept beehives in an enclosed area. Hanging and keeping beehives in the dense forest which are mostly far away from residential areas might have limited beehive visit to only one or two times until harvesting and unawares about the condition of the site.

Table 3 – Placement of hive(%) by sample households

Placement of beehive	Fiq (n=78)	Lagahida (n-78)	Overall (n=156)
Hanging on trees	69.2	76.9	73.1
Backyard	18	16.7	17.3
Both	12.8	6.4	9.6

Honeybee flora

Table 4 lists some of the major honeybee floras in the study area, along with their botanical names. According to the sampled respondents, the major honey bee floras were Dhafaruur, Hobhob, Bilcil (*Acacia mellifera*), Qansax (*Acacia reficiens*), Qudhac (*Acacia tortilis*), Cadaad (*Acacia senegal*), and Sarmaan (*Acacia horrida*). According to the study, the honeybee flora is primarily made up of trees and shrubs which provide nectar and pollen. This variation in vegetation characteristics of the area could be significant enough to ensure adequate pollen and nectar for bee reproduction and honey production.

Table 4 – Major honeybee flora (%) in the study area

Local name	Botanical name	Type	Rank
Dhafaruur	Grewia tenax	Shrub	1
Hobhob	Grewia penicillata	Shrub	2
Bilcil	Acacia mellifera	Tree/wood	3
Qansax	Acacia reficiens	Wood	4
Qudhac	Acacia tortilis	Tree/wood	5
Cadaad	Acacia Senegal	Tree/wood	6
Sarmaan	Acacia horrida	Tree/wood	7

*honeybee flora were ranked based on the number (frequency of respondents' prioritization and availability

Water availability

River and nearby ponds, which are primarily used during the dry season, were the primary water sources for honeybees in the study area. Beekeepers did not provide water for bees near their apiaries. Furthermore, the majority of respondents reported that there was a scarcity of water in the area, particularly during the dry season, which is one of the key constraints of beekeeping in the study area.

Honey production

Table 5 shows the average annual honey yield per hive from traditional and modern bee hives in Fiq and Lagahida districts. The average honey yield from the traditional beehives was significantly higher (P<0.05) for Fiq district (5.10±0.61) kg//hive and lower for Lagahida district (4.60±0.65) kg/hive. The average honey yield from modern beehives, on the other hand, was slightly higher (P<0.05) for Fiq (8.58±1.69) kg/hive and lower for Lagahida (8.00±1.66) kg/hive. The variation in average annual honey yield per hive from traditional beekeeping in the localities was attributed to differences in hive volume and beekeeper skill. The honey value obtained from traditional hives in the current study (4.85 kg) is less than the national average yield (5 kg). Yirga et al. (2012) reported that productivity and overall production increases with the level of management, experience and area potentiality.

The honey obtained from modern hives in the current study (8.29 kg) was higher than the 7.14 kg found by Fikru et al. (2015) for the Jigjiga zone of the Somali region. However, it was significantly lower than the average yield of 20 and 22

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kg in Keffa zone, SNNPRS and in Jimma and Illubabor Zone of Oromia Regional State, respectively (Kiros and Tsegay, 2017; Getachew, 2018).

Table 5 –Honey yield (kg) in the study area (Mean±SD) by sampled households

Hive type	Fiq	Lagahida	Overall	*P*-value
Traditional hive	5.10±0.61[a]	4.60±0.65[b]	4.85±0.68	0.001
Modern hive	8.58±1.69 [a]	8.00±1.66 [b]	8.29±1.70	0.03

*Means with different letter of superscripts in the same column are different significantly at *P*<0.05

Honey harvesting and marketing

According to the information collected from the respondents, honey is harvested two times per year and the price of honey in the area varied from 250 to 300 ETB/kg. However, the price of honey fluctuates with highest price in the dry season (January to April), and also during wet season (June to August) in the period when there was no honey production and lowest price during honey harvesting time (September to November and May). The general marketing of honey in the area was promising. They use honey as medicine, food and drinks. Almost all interviewed beekeepers did not harvest bee wax because of lack of awareness about the product.

According to respondent beekeepers and our observations at various levels, lack of appropriate hive products' marketing place, lack of market information, absence or lack of known market route or channel, buyer dependent price settings, lack or inappropriate functioning of marketing cooperatives, less awareness on post-harvest handling of their products have been identified as major constraints in the marketing system.

Major constraints of beekeeping

According to the information collected from the respondents, honey is harvested two times per year and the price of honey in the area varied from 250 to 300 ETB/kg. However, the price of honey fluctuates with highest price in the dry season (January to April), and also during wet season (June to August) in the period when there was no honey production and lowest price during honey harvesting time (September to November and May). The general marketing of honey in the area was promising.

According to respondents, the major constraints of beekeeping are the high cost of modern hives and accessories, pests and predators of honey bees, water scarcity, shortage of bee forage, bee absconding and marketing. The present study is supported by Yirga et al. (2012), who reported that bee pests, predators and absconding are major constraints affecting beekeeping sub-sector in northern Ethiopia. The current study is also consistent with Fikru et al. (2015), who reported that during the field survey, the interviewed beekeepers in Jigjiga zone responded that some bee equipment, such as modern bee hives, wax printers, and honey extractors, are very expensive, and thus farmers cannot afford to buy and use these equipment. As a result, there is a scarcity of appropriate technologies for production, collection, processing, packing, and storage in the area. Because the majority of the farmers in the study area lacked resources, they were unable to purchase and implement modern bee technologies to increase honey yield.

Table 6 –Major constraints of beekeeping in the study area

Constraint	Rank	Measures
Beekeeping equipment	1	Use of locally available materials
Pests and predator	2	Hive cleaning
Water scarcity	3	Preparing water ponds
Shortage of bee forage	4	Increasing bee forage by cultivating different crops
Absconding of bees	5	Overall management practice
Marketing	6	Harvest and store

*Constraints were ranked based on the number (frequency) of respondents prioritize the problems

Pests and predators

Honey bees, like all living organisms, are vulnerable to pests and predators at all stages of their life cycle. The study discovered that the presence of pests and predators was a major challenge in beekeeping. Birds, ants, spiders, wax moth, mice, lizards, and honey badgers were identified as the major honeybee pests and predators based on beekeeper responses. The study is substantiated by Fikru et al. (2015) who reported that mites, spider, bee-eater birds, lizard etc. are the most serious problems to beekeeping development in Jigjiga zone, Ethiopia. Furthermore, Amsalu (2020) identified ants, beetles, wax moth, honey badger, bee-eater birds, dead hawks moths, bee lice and some predators like, lizards, wasps and spiders as the major pests and predators considered as challenges ranked with their relative degree of importance. Similarly, Getachew (2018) reported that ant attack, honey badger, birds, wax moth, lizard, and wax moth are bee pests and enemies in Ethiopia that affect bees and beekeeping. Similarly, Shenkute et al. (2012) reported that

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ants, honey badgers, birds, and small hive beetles are the most common honeybee enemies in the Keffa, Sheka, and Bench-Maji zones. The author also stated that honeybee enemies are responsible for significant losses (40.7%) of total honey production per year. This indicates that honey bee pests and predators have a significant impact on beekeeping households' income.

Absconding of bee colonies

The study revealed that most of the respondents experienced absconding of bee colonies. Various causes of honeybee absconding were identified in the present study (Table 7). According to the respondents, the main reasons for absconding were due to pests and predators (37.8%), destroying nests during harvesting (26.9%), shortage of bee forage (14.1%), and water scarcity (21.2%). The findings of this study are consistent with those of Getachew (2018) and Kinati et al. (2012), who reported similar reasons for bee colony absconding in the Gesha and Goma districts, respectively. Similarly, Shibru et al. (2014) reported that honeybee absconding and migration as the main constraints of beekeeping in Gambella Zuria and Godere Wereda of Gambella Regional State, Ethiopia.

The presence of honey bee pests and predators resulted in frequent colony absconding and high migratory tendencies. During honey harvesting from traditional hives, beekeepers dismantle the hive, damage the brood, and abandon the colony, resulting in the colony's eventual disappearance. This is consistent with the findings of Shenkute et al. (2012), who reported similar results in the Keffa and Sheka zone. Due to shortage of bee forage, the honeybee colony migrates to areas where resources are available for survival. Bee forage scarcity is directly related to the off-flowering period of major honeybee plants. About 73.7% of the interviewed households stated that improving overall management (feeding bees, cleaning the hive, appropriate harvesting technique, etc.) as a method to avoid absconding. On the other hand, 26.3% of respondents believed absconding was unavoidable and thus did nothing to prevent it. These farmers believed that when the colony was disturbed for honey harvesting, the bees would flee and never return to the hive.

Table 7 – Causes of honeybee absconding (%) in the study area

Variables	Fiq (n=78)	Lagahida (n-78)	Overall (n=156)
Causes of absconding			
Pests and predators	35.9	39.8	37.8
Shortage of bee forage	16.7	11.5	14.1
Destroying of nests	28.2	25.6	26.9
Water scarcity	19.2	23.1	21.2
Control mechanism of absconding			
Overall management	76.9	70.5	73.7
Do nothing	23.1	29.5	26.3

Opportunities of beekeeping

Even though different constraints have been described for their possible effects exerted on the beekeeping subsector in the area, it has been an established fact that the area is endowed with different opportunities and immense potentials. Accordingly, some of the opportunities associated with the study area and described by the respondent beekeepers included increasing hive products' demand, availability of honeybee floral resources, and availability of honeybee resources.

CONCLUSION

Beekeeping has been practiced as a sideline activity in the area by many rural farming communities as an income generation and livelihood activity. The prevailing honey production system in the study area is based on traditional beekeeping technique dominated by forest beekeeping which compromises the quantity and quality of honey. Furthermore, various constraints including pests and predators, absconding of bees, shortage of bee forage, water scarcity, and lack of appropriate knowledge to manage bees have hampered honeybee production in the area. Furthermore, Ants, honey badger, birds, spider, wax moth, and lizards are the major damaging bee pests in the area. Beekeeping production remains low due to these constraints and traditional practices in the area. Despite the numerous constraints, it is impossible to ignore the activity due to its contribution to the livelihood and food security of the farming community, as well as its vital role in maintaining the natural resources of the area. Moreover, there is enormous potential to increase the amount and quality of honey yield in the area in order to improve the communities' livelihoods in a sustainable manner. Therefore, all stakeholders in the area should collaborate for the improvement and development of the sub-sector in order to benefit farmers in particular and the country as a whole by raising awareness about good management practices of honeybee, introducing local made or least cost technologies and as well as the harmful effects and control measures of pests and predators.

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DECLARATIONS

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Authors' contribution

The author contributed on data collection, analysis and the write up of the manuscript as well.

Conflict of interests

The authors have not declared any conflict of interests.

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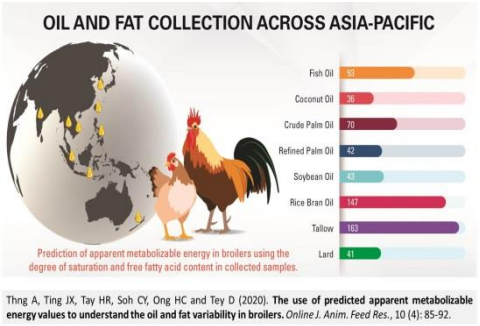

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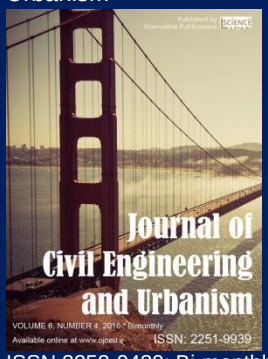

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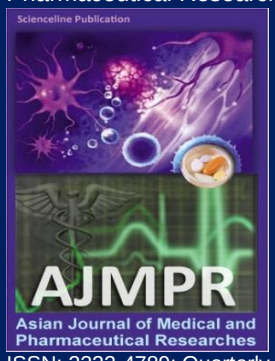

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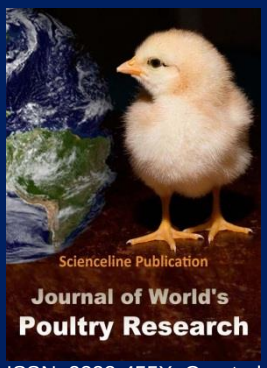

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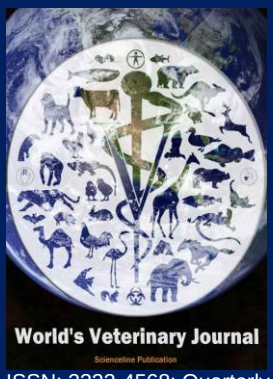

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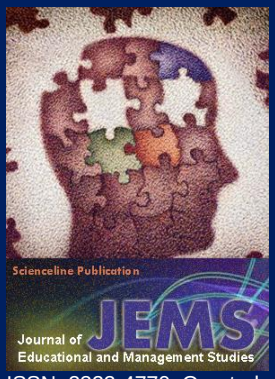

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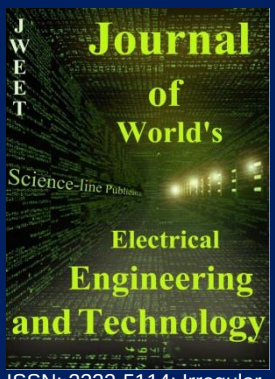

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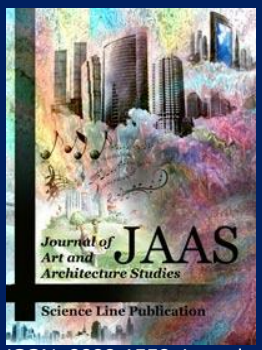

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