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Volume 9 (1); January 25, 2019**Research Paper****Value chain and evaluation of milk quality in selected districts around Addis Ababa, Ethiopia.**

Etifu Meranga M and Abu Kufa M.

Online J. Anim. Feed Res., 9(1): 01-12, 2019; pii: S222877011900001-9**Abstract**

Evaluation of milk value chain and the quality of milk were assessed in selected districts of surrounding Addis Ababa from September 2016 to April 2017. A total of 180 randomly selected market-oriented smallholder dairy farmers were involved in a cross-sectional study that was carried out by way of a questionnaire survey, rapid market appraisal, farm inspection and group discussion. The overall mean family size of respondents in this study was 5.63 ± 1.926 persons with average livestock holding per household of 23.93 ± 11.755 animals. Cattle were the predominant species representing 84.3% of the total TLU. The average number of lactating cows owned by the respondent farmers was 1.76 ± 0.920 local and 2.79 ± 3.445 crossbred animals. Average daily milk yield of crossbred and local milking cows were 9.11 ± 2.902 and 1.889 ± 0.6707 liters respectively. Overall mean lactation lengths of crossbred and local milking cows were 9.7 ± 0.46 and 6.26 ± 0.6624 months. Sixty milk samples were collected and microbiological and physicochemical analyses were carried out. The overall mean chemical compositions of milk for fat, protein and solids not fat (%) were 3.5693 ± 0.10892 , 2.9646 ± 0.04621 and 6.9632 ± 0.12175 in bulk Tank milk samples. The overall mean microbiological count of log (TBC cfu/ml), log (CC cfu/ml) and log (SCC/ml) of raw milk was 8.2285 ± 0.10041 , 3.3363 ± 0.10010 and 5.1622 ± 0.07382 , respectively. The proportion of raw milk used for household consumption was relatively small (5%). The major part (86%) of milk produced by smallholders is destined to market. The main outlets for raw milk identified were cooperatives (55.6%), processors (20.0%), vendor (20.0%), directly to consumer (2.8%) and hotels/ restaurants (1.7%). Price variations (cited by 87% of the respondents), lack of fair market (72.2%), lack of demand during fasting (49.4%), lack of preserving facilities, and absence of quality-based payment and no/less say in deciding milk price by producers were the major problems of raw milk marketing.

Keywords: Coliform, Milk, Physicochemical, Total bacteria, Value-chain.



Etifu Meranga M and Abu Kufa M (2019). Value chain and evaluation of milk quality in selected districts around Addis Ababa, Ethiopia. *Online J. Anim. Feed Res.*, 9(1): 01-12. www.ojafir

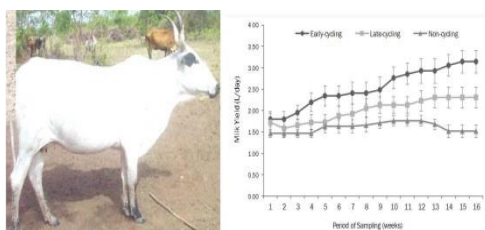
[\[Full text-PDF\]](#)**Research Paper****Impact of body condition, milk yield and blood metabolite concentrations on the resumption of ovarian activity in cattle.**

Obese FY and P Tecku.

Online J. Anim. Feed Res., 9(1): 13-19, 2019; pii: S222877011900002-9**Abstract**

Body condition of cows, level of milk production and concentrations of blood metabolites can influence the resumption of ovarian activity in cattle. This study investigated the relationships among body weight (BW), body condition score (BCS), milk yield, blood metabolite [Glucose, total protein, albumin, globulin, urea, triglyceride, non-esterified fatty acids (NEFA) and Beta-hydroxybutyrate (BHB)] concentrations and resumption of postpartum ovarian cyclicity in 20 Sanga and 20 Friesian \times Sanga cows. Cows were classified as having resumed ovarian activity when they had plasma progesterone concentration of ≥ 1 ng/mL. Based on the resumption of ovarian activity, cows were also classified into three ovarian cyclicity groups namely; early resumption of ovarian cyclicity (≤ 56 days postpartum), late resumption of ovarian cyclicity (57-112 days postpartum) and non-resumption of ovarian cyclicity (by 112 days postpartum). BW, BCS and milk yield did not affect ($P < 0.05$) cycling status in Sanga cows. Friesian \times Sanga that resumed ovarian cyclicity early (2.54 L/day) or late (2.01 L/day) had higher milk yield ($P < 0.05$) than their counterparts that did not resume ovarian cyclicity during the period of study (1.61 L/day). However, BW and BCS were similar ($P > 0.05$) in the three ovarian cyclicity groups in the Friesian \times Sanga cows. Sanga cows that resumed ovarian cyclicity early had lower total protein concentration than those that resumed ovarian cyclicity late (76.8 versus 89.3 g/L; $P < 0.05$). Globulin concentration was also lower ($P < 0.05$) in cows that resumed cyclicity early (42.6 g/L) than late (54.2 g/L) and non-cycling (49.7g/L) Sanga cows. There were significant ($P < 0.05$) positive and negative correlations among some of the plasma metabolites determined. In conclusion, earlier resumption of ovarian cyclicity was associated with higher milk yield in Friesian \times Sanga cows and lower concentrations of total protein and globulin in Sanga cows. Improved feeding and management strategies are recommended for the achievement of reproductive success in cows.

Keywords: Body Weight, Cow, Nutritional Status, Ovulation, Relationships, Supplement



Obese FY and P Tecku (2019). Impact of body condition, milk yield and blood metabolite concentrations on the resumption of ovarian activity in cattle. *Online J. Anim. Feed Res.*, 9(1): 13-19. www.ojafir

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Research Paper

Diagnosis of subclinical endometritis during postpartum period on subsequent pregnancy in small, medium and large scale dairy farms in and around Gondar, Ethiopia.

Moges N.

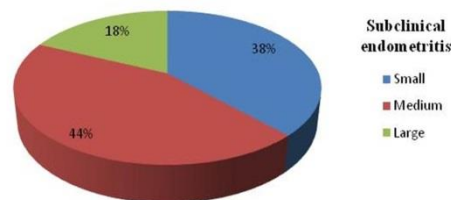
Online J. Anim. Feed Res., 9(1): 20-25, 2019; pii: S222877011900003-9

Abstract

During the study period 147 apparently healthy 3rd trimester pregnant cows were selected in smallholder, medium and large scale dairy farms in and around Gondar, North Western Ethiopia from January 2015 to September 2017. After calving, endometrial samples were collected from the uterus of apparently normal cows by using uterine lavage technique on postpartum dairy cows from 40-60 days. Collected samples were centrifuged and a drop of sediment was streaked onto a clean microscopic slide and stained with giemsa. The percentage of polymorphonuclear cells (neutrophils) was calculated. It was found that increase in the number of neutrophils correlated with decrease in pregnancy. In conclusion, subclinical endometritis diagnosed by endometrial cytology was associated with reduced rate of pregnancy.

Keywords: Subclinical Endometritis, Endometrial Cytology, Pregnancy, Neutrophiles

[Full text-PDF]



Moges N (2019). Diagnosis of subclinical endometritis during postpartum period on subsequent pregnancy in small, medium and large scale dairy farms in and around Gondar, Ethiopia. *Online J. Anim. Feed Res.*, 9(1): 20-25. www.ojafri.ir

Research Paper

Effect of hatch window upon intestinal development, chick quality, post hatch performance according to Ross-308 broiler breeder age.

Yousaf A, Rajput N, Memon A, Naz Jagirani G, Shahnawaz R, Rajpar S, Sarwar M and Habib F.

Online J. Anim. Feed Res., 9(1): 26-32, 2019; pii: S222877011800004-9

Abstract

This study was carried out to find out the effects of breeder age on incubation results, intestinal development during hatch window, chick quality and first week broiler performance. A total of 259280 eggs were obtained from commercial Ross-308 broiler breeder flocks at 32 and 52 weeks old, which were equally divided on the basis of breeder age in two groups, group A (young 32 weeks) and group B (old 52 weeks age). These 32 and 52 weeks old flocks, 30% and 7.0% were hatched 24h before pull time and 50% and 60% were hatched 12h before pull time, respectively. Hatchability of fertile eggs and hatchability of total eggs were found higher in 32 weeks old flock than the other. The chick hatch weight was determined as 39.5g and 42.1g in 32 and 52 weeks old flocks, respectively. Chick weight/initial egg weight rate was found to be higher as 67.3% in the 32 weeks old flocks. On hatching day, chick length was also higher in 52 weeks old flock. Relative yolk-free chick weight was higher in 32 weeks old (86.0%) flock than the other (83.0%). Intestine weight rate was higher as 5.24% in chicks from 32 weeks old than the other (4.96%). At one week of age, the body weights and weight gains were 167.5g and 162.7g, and 128g and 120.6g in 32 and 52 weeks old flocks, respectively. Higher mortality ratio as 1.9% was observed in 52 weeks old flock. In conclusion, intestinal development during hatch window, incubation parameters, chick quality and first week broiler performance is affected by breeder age.

Keywords: Incubation duration, Intestinal development, Hatch window, Post-hatch performance, Ross-308 broiler breeder

[Full text-PDF]



Yousaf A, Rajput N, Memon A, Naz Jagirani G, Shahnawaz R, Rajpar S, Sarwar M and Habib F (2019). Effect of hatch window upon intestinal development, chick quality, post hatch performance according to Ross-308 broiler breeder age. *Online J. Anim. Feed Res.*, 9(1): 26-32. www.ojafri.ir

Research Paper

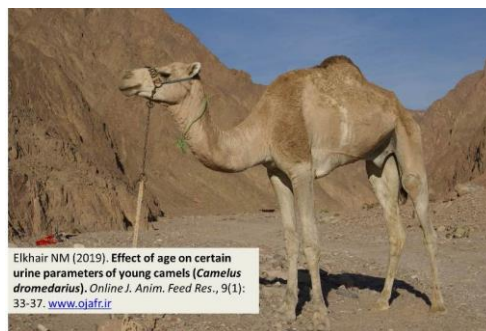
Effect of age on certain urine parameters of young camels (*Camelus dromedarius*).

Elkhair NM.

Online J. Anim. Feed Res., 9(1): 33-37, 2019; pii: S222877011800005-9

Abstract

The objective of this study was to identify reference values for certain urine parameters in relation to the age in young dromedary camels. Twenty one healthy young female camels (age <3-5 months) of Arabi local breed were divided into three groups (≤ 3 m: n=6, 3-4 m: n=8 and 4-5 m: n=7). Jugular venous blood samples were collected and used to determine electrolytes and creatinine concentration, whereas the urine samples were used for the determination of urine pH, urine osmolality, and for the calculation of fractional excretion of electrolytes. The mean value of urine pH was 7.9 ± 0.5 (reference range: 7.2-8.8 and the percentile range: 7.3-8.2) and the urine osmolality in young camels was 978.9 ± 468 (reference range 235-1819 mOsmol/kg) for all age groups. Fractional excretion of Na^+ (FE_{Na^+} %) showed lower mean values compared to the fractional excretion of K^+ and Cl^- . The age had a significant ($P < 0.05$) effect only on urine osmolality in camels of 4-5 months. The identified reference values of certain urine parameters can be used for the clinical diagnosis of renal diseases



Elkhair NM (2019). Effect of age on certain urine parameters of young camels (*Camelus dromedarius*). *Online J. Anim. Feed Res.*, 9(1): 33-37. www.ojafri.ir

in growing animals. The data could be utilised for the clinical monitoring of the physiological and pathological status of animals fed strong electrolytes such as Na⁺, K⁺ and Cl⁻.

Keywords: Age, Dromedary camels, Reference values, Urine parameters

[Full text-PDF]

Research Paper

The influence of feed form on broiler production and gastrointestinal tract development.

Kuleile N and Molapo S.

Online J. Anim. Feed Res., 9(1): 38-43, 2019; pii: S222877011800006-9

Kuleile N and Molapo S (2019). The influence of feed form on broiler production and gastrointestinal tract development. *Online J. Anim. Feed Res.*, 9(1): 38-43. www.ojafri.ir



Abstract

A completely randomized design experiment was conducted to assess the influence of feed form on broiler production and gastrointestinal tract development. A total of (n= 315) 16 days-old Ross 308 chicks were randomly assigned to three dietary treatments (mash, crumbles and pellets) with three replicates. Weekly data indicated that birds on pellet and crumble diets had a significant ($P < 0.05$) improvement on a number of production parameters than birds fed diet in a mash form. Data for average values for the whole experimental period demonstrated that the dietary treatments had significant effect on feed intake (0.001), body weight ($P= 0.013$), performance index ($P=0.040$), abdominal fat weight ($P= 0.010$) and carcass weight. ($P= 0.001$). However, feed conversion ratio and carcass yield did not show significant ($P > 0.05$) response to dietary treatments. Contrary to weekly data the averages for the whole experimental period data was dominated by birds on crumble diet with the highest feed intake, body weight, abdominal fat weight and carcass weight. Gastrointestinal tract development results indicated that dietary treatment had a significant effect on intestinal length ($P= 0.015$) whereby birds that consumed diet in a mash form had superior intestinal length. The intestinal and gizzard weight parameters were not significantly ($P > 0.05$) influenced by the dietary treatment. The results of the current study proved that the crumble form of a diet is the most suitable for optimum broiler production during growing and finishing stages while diet in a mash form is suitable for optimum development of gastrointestinal tract.

Keywords: carcass yield, Crumble, Feed form, GIT development, Mash, Pellets

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VALUE CHAIN AND EVALUATION OF MILK QUALITY IN SELECTED DISTRICTS AROUND ADDIS ABABA, ETHIOPIA

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✉ Supporting Information

ABSTRACT: Evaluation of milk value chain and the quality of milk were assessed in selected districts of surrounding Addis Ababa from September 2016 to April 2017. A total of 180 randomly selected market-oriented smallholder dairy farmers were involved in a cross-sectional study that was carried out by way of a questionnaire survey, rapid market appraisal, farm inspection and group discussion. The overall mean family size of respondents in this study was 5.63 ± 1.926 persons with average livestock holding per household of 23.93 ± 11.755 animals. Cattle were the predominant species representing 84.3% of the total TLU. The average number of lactating cows owned by the respondent farmers was 1.76 ± 0.920 local and 2.79 ± 3.445 crossbred animals. Average daily milk yield of crossbred and local milking cows were 9.11 ± 2.902 and 1.889 ± 0.6707 liters respectively. Overall mean lactation lengths of crossbred and local milking cows were 9.7 ± 0.46 and 6.26 ± 0.6624 months. Sixty milk samples were collected and microbiological and physicochemical analyses were carried out. The overall mean chemical compositions of milk for fat, protein and solids not fat (%) were 3.5693 ± 0.10892 , 2.9646 ± 0.04621 and 6.9632 ± 0.12175 in bulk Tank milk samples. The overall mean microbiological count of log (TBC cfu/ml), log (CC cfu/ml) and log (SCC/ml) of raw milk was 8.2285 ± 0.10041 , 3.3363 ± 0.10010 and 5.1622 ± 0.07382 , respectively. The proportion of raw milk used for household consumption was relatively small (5%). The major part (86%) of milk produced by smallholders is destined to market. The main outlets for raw milk identified were cooperatives (55.6%), processors (20.0%), vendor (20.0%), directly to consumer (2.8%) and hotels/ restaurants (1.7%). Price variations (cited by 87% of the respondents), lack of fair market (72.2%), lack of demand during fasting (49.4%), lack of preserving facilities, and absence of quality-based payment and no/less say in deciding milk price by producers were the major problems of raw milk marketing.

Keywords: Coliform, Milk, Physicochemical, Total bacteria, Value-chain.

INTRODUCTION

Ethiopia has a huge potential to be one of the key countries in dairy production for various reasons (Pratt et al., 2008). These include a large population of milk cows in the country estimated at 9.9 million (CSA, 2008), a conducive and relatively disease free agro-ecology, particularly the mixed crop-livestock systems in the highlands that can support crossbred and pure dairy breeds of cows (Ahmed et al., 2003), a huge potential for production of high quality feeds under rain fed and irrigated conditions, existence of a relatively large human population with a long tradition of consumption of milk and milk products and hence a potentially large domestic market (Holloway et al., 2000).

A number of fundamental constraints underlie these outcomes, including traditional technologies, limited supply of inputs (feed, breeding stock, artificial insemination and water), poor or non-existent extension service, high disease prevalence, poor marketing infrastructure, lack of marketing support services and market information, limited credit services, absence of effective producers' organizations at the grass roots levels, and natural resources degradation (Berhanu et al., 2006). In addition, policy decision on milk and milk product marketing are taken in the absence of vital information on how they affect dairy producers, traders, exporters, and consumers. Similarly, current knowledge on dairy product market structure, performance and prices is poor for designing policies and institutions to overcome the perceived problems in the marketing system (Ayele et al., 2003).

Traditional farmers sell their raw milk informally due to the absence of organized marketing network that has made the produced milk unable to reach the consumer. Further losses incurred are quality losses by storing in unclean storage utensil, which is prone to high microbial contamination. Losses in spillage and contamination occur where handling during and after milking is traditional and care is not satisfactory. Additionally, the trade in the sub-sector is constrained by various structural, production, information exchange, and promotional problems, as well as financial constraints.

Therefore, this study was conducted to evaluate value chain and the quality of milk in selected districts around Addis Ababa.

MATERIALS AND METHODS

This study was conducted in purposively selected districts around the capital city of Addis Ababa namely Welmera and Sululta which are known for smallholder dairies are practiced for distribution of milk and milk products to the nearby city Addis Ababa. Welmera district is located 28 km west of Addis Ababa at 09°02' North latitude and 38°34' East longitudes with its altitude ranging from 2060-3380 m.a.s.l. whereas Sululta district lies between 39°30' N Latitude and 38° 30' and 39° 00' E longitude. It is located 40 km north west of Addis Ababa.

Study population

Smallholder farmers in Sululta and Welmera districts owning crossbred and indigenous cattle for milk production constituted the study population.

Study design

A cross-sectional study by way of questionnaire survey, rapid market appraisal, farm inspection, group discussion, interviewing key respondents and laboratory analysis of raw milk samples was carried out from September 2016 to April 2017. Marketing actors and smallholder dairy farmers in the selected study area were study participants.

Sample size determinations

The sample size was determined by using mathematical model of Arsham (2007). The sample size, N, can then be expressed as largest integer less than or equal to $0.25/SE^2$.

$$N=0.25/SE^2$$

Where, confidence level of 95% and confidence interval of 5%, were considered. Based on the above formula the computed sample size was 180.

Sampling procedure

To select a representative sample, the potential of the two districts were identified. Sululta district has 23 peasant associations (PAs), of which eight have potential in dairy production. From the list of these eight PAs, three were selected randomly. These included Moye-Gajo, Chancha-Buba, and Warrarso-Malima PAs. Then ninety households owning dairy cattle were selected randomly from three PA's (thirty from each PA). Welmera District has also 24 PAs, of which six have potential in dairy production. From the list of these six, three of them namely Gelgelikuyu, Bekeka na kore-oddo and Gebarobi PAs were selected. Then ninety households owning dairy cattle were selected randomly from the three PA's.

Data collection

Questionnaire survey. The questionnaire that was structured and closed type for its major part was pre-tested before its full administration. The questionnaire was focusing on demographic characteristics of the study participants, husbandry practices, milk production, processing, and marketing and utilization situations. Furthermore, marketing constraints of raw milk was investigated.

Rapid Market Appraisal (RMA). Rapid Market Appraisal (RMA) using checklists and observation was implemented to understand how a product or commodity flows to reach the end users.

Farm inspection. Farms were inspected once at the same time with the questionnaire survey. Activities observed during the farm visit encompassed kinds of utensils used, milking practices, milk handling and storage conditions.

Group discussions. Group discussions at three different PA'S of Sululta and three PA'S of Welmera were undertaken, in order to understand the overall community situations and get insight about milk marketing, milk handling, limitations and strength milk marketing. Groups were composed of 10 to 12 members constituted by different age and social groups. Discussion participants were identified in consultation with the district development agents. A sample checklist, which served as a guide and consisting of the main points for the group discussion was prepared.

Interviewing key respondents. Chairmen of PA'S, representatives of the sub PA'S and extension workers were interviewed. The agricultural office workers at PA'S levels were also participants in the process.

Collection of raw milk samples. Raw milk samples were collected at farm and milk collections centers by following strict aseptic procedures. Physicochemical test of raw milk was performed and the presence of bacteriological agents was assessed; standard plate count, coliform and somatic cell count tests were done. Before

sampling, milk was thoroughly mixed after which 25 ml of milk was transferred into sterile sampling bottles. The milk sample bottles were capped, labeled with a permanent marker and stored in an ice packed cool box and transported to the Ethiopian Meat and Dairy Technology Institute, Debre-zeit where the different analysis were conducted.

Bacteriological quality tests

Tests employed to determine the quality of milk were Standard plate count, Coliform count, and Somatic cell count. A detailed description of the steps followed in each of the methodologies is presented in the following sections.

Standard plate count (SPC)

The standard plate count of raw milk samples was performed by putting one ml of milk sample into a sterile test tube having 9 ml peptone water. After mixing, the sample was serially diluted up to 1: 10⁻⁷ and duplicate samples of 1 ml of diluted milk samples were streaked on 15-20 ml standard plate count agar media and then incubated for 48 hours at 37°C to encourage bacterial growth. Finally, colonies counts were made using colony counter. Single bacteria species or clusters grow to become visible colonies that were then counted. All plate counts were expressed as the number of colony forming units (CFU) per milliliter. Results from plates, which contained 10 to 300 colonies per plate were recorded. If plates from two consultative decimal dilutions yield colony counts of 10 to 300, the counts for each dilution were computed by the following formula (APHA, 1993).

$$N = \frac{\sum \text{colonies}}{[(1 \cdot n_1) + (0.1 \cdot n_2)] \cdot d}$$

Where: N = number of colonies per milliliter of milk,

∑C = sum of colonies on plates counted,

n₁ = number of plates on lower dilution counted,

n₂ = number of plates in next higher dilution counted and

d = dilution from which the first counts are obtained.

Coliform count (CC)

One ml of milk sample was added into a sterile test tube having 9 ml peptone water. After mixing, the sample was serially diluted up to 1: 10⁻⁴ and duplicate samples (1 ml) were pour plated using 15-20 ml Violet Red Bile Agar solution (VRBA). After thoroughly mixing, the plated sample was allowed to solidify and laying over by Violet Red bile Agar solution (VRBA) then incubated at 37°C for 24 hours. Finally, colony counts were made using colony counter. Typical dark red colonies were considered as coliform colonies.

Somatic cell count (SCC)

For counting somatic cells, the microscopic method was used. Milk film preparation, staining and counting were done according to the standards set by the International Dairy Federation (IDF, 1995). To obtain a uniform distribution of cells, milk samples were mixed by moving upside down gently 25 times and letting it to stand for 2 minutes to permit air bubbles and foam disappear. Microscopic slides were degreased with alcohol before milk film preparation. A 0.01ml of milk was taken with a 50µl micropipette calibrated at 10 and spread evenly over one cm² area on a microscopic slide and allowed to dry at room temperature on a leveled table. One cm² area was delineated by a template prepared from a cap board. Dried films were fixed with ethanol for 15 minutes. Stained with toluidine blue for 5 minutes and washed with tap water gently and allowed to dry in a dust free area. Stained slides were stored in slide box until counted. Using oil immersion objective those cell nuclei clearly recognizable and those at the periphery with more than 50% of the cell body in view were counted. Twenty fields were counted from given sampled milk. The number of cells per ml of milk was calculated by multiplying the average number of cells per field with Magnifications filed (laboratory manual).

$$\text{Somatic cell per ml of milk} = \frac{\sum \text{SCC per field} \cdot 10,000}{0.0346 \cdot 20}$$

Where ∑SCC per field = the summations somatic cell counted per each field

0.0346 = oil immersion calibrated

20 = Total number of field counted

Physicochemical test

The chemical compositions of milk (fat, protein, and solid not fat) and physical characteristics (density and freezing point), of the milk samples, were determined by Eko-milk analyzer (Bulgaria), according to manufacturer's instructions. Milk samples were mixed gently 4-5 times to avoid any air enclosure in the milk. Then 25 ml samples were taken in the sample-tube and put in the sample- holder one at a time with the analyzer in the recess position.

Then when the starting button activated, the analyzer sucks the milk, makes the measurements, and returns the milk in the sample-tube and the digital indicator (IED display) shows the specified results.

Data analysis

The data collected were entered into Micro-soft-Excel spreadsheet for managing the data and analyzed using SPSS version 17. Descriptive statistics like means, standard deviation and frequency distribution were used to describe the farming system characteristics in the study area. One-way ANOVA statistical analysis was used for comparison of the performance variation. The correlation statistical analysis was used to study the interaction between the farming system characteristics and the interaction between physicochemical and microbiology of raw milk sample.

RESULTS

The overall mean family size for all respondents was 5.63 ± 1.926 persons. The family size ranged from 2 to 12 people. Fifty-two percent of the family members were male and the rest (48%) were female. The overall average livestock holding per household was 23.93 ± 11.755 . The average family and herd size of the two districts namely Sululta and Wolmera pointed out by the respondents (Table 1).

Cattle composition

Table 2 shows the size and composition of cattle owned by the smallholders in the study areas. All the surveyed smallholders owned on average 13.03 ± 8.802 (12.29 TLU) cattle. The average number of Lactating cows owned by the respondent farmers was 1.76 ± 0.920 local or 1.76 TLU and 2.79 ± 3.445 crossbred animals or 4.185 TLU. Cattle were the predominant species representing 84.3% of the total TLU. The smallholders prefer to have crossbred cows because of their greater milk production, even though they require high management and susceptible to disease than local breeds.

Milking and milk handling practices

Ninety-four percent of the respondents of the study area were using the plastic pail for milking and milk handling. Nearly 6% were using Stainless steel pail. Difficulties of using these utensils were difficult for cleaning (1.1%), accessibility in local markets (5%) and no problem of using these utensils (93.9%) were indicated by the respondents of the study area. Through group discussions with the participant of the study areas it was pointed out that all the respondents practice washing the utensils used for milking and milk handling. Commonly they were washing the milking utensils with warm water by using soap and finally allow drying till milking. In the study area cows are hand milked and calves are allowed to suckle their dams prior to as well as after milking. About 100% of the respondents in Sululta and Wolemera area pointed out that they milk their cows two times a day at morning and evening. They milked their cows at the barn, where the animals are sheltered. As illustrates on Table 3, all respondents were washing their hands and vessels before milking. Seventy-two percent of respondents were also washing udder before milking. Nearly 19% of the smallholders were using individual towels for cleaning udder of milking cows, in 52.2% of the cases collective towels were used while in the rest (28.9%) no towel use was practiced.

Milk production and use aspects

Mean of Lactation length of crossbred and local milking cows were 9.72 ± 0.45 and 6.353 ± 0.7681 in Sululta, 9.68 ± 0.47 and 6.167 ± 0.5567 in Wolmera district respectively and overall mean of lactation length of crossbred and local milking cows were 9.7 ± 0.46 and 6.26 ± 0.6624 months respectively. Average daily milk yield of cross bred and local cows in Sululta were 9.56 ± 3.010 and 1.809 ± 0.4574 liter/day respectively. Moreover, crossbred and local cows in Wolmera areas were 8.60 ± 2.703 and 1.96 ± 0.8193 liters/day respectively. Overall mean summery of daily milk yield at the study areas of crossbred milking cows (9.11 ± 2.902) and local milking cows (1.889 ± 0.6707) liters as shown on table 4.

Overall mean of milk producing, Processing, consuming and selling per day per household was 26.88 ± 4.76 , 1.23 ± 1.603 , 1.29 ± 1.176 and 23.32 ± 5.22 liters respectively (Table 5). The proportion of raw milk used for household consumption was relatively small. As figure 1 illustrates, the major part of milk produced by smallholders is destined to market. Smallholders also process milk to butter and cheese. Milk was soured for 2-3 days before processing it in to butter and cheese. The one way of ANOVA analysis showed significance difference at ($P < 0.01$) and ($P < 0.05$) among the District from which the milk sample for milk produced and milk sold per day/liter.

Table 1 - Average family size and herd in smallholder dairy farms in the study district.

Variables	Sululta (N=90) Mean±SD	Wolmera (N=90) Mean±SD	Overall (N=180) Mean±SD
Family size	5.49±1.819	5.77±2.028	5.63±1.926
Male	2.88±1.211	3.04±1.469	2.96±1.346
Female	2.68±1.198	2.76±1.248	2.72±1.220
Livestock	25.22±12.382	22.63±11.009	23.93±11.755
Cattle	14.69±11.619	11.37±3.905	13.03±8.802
Lactating cows	4.57±4.316	3.38±1.427	3.97±3.260
Local cows	2.18±0.384	2.08±.278	2.13±0.336
Cross bred cow	4.28±0.450	4.32±.470	4.30±0.459
Sheep	5.92±4.238	6.66±4.490	6.26±4.356
Equines	1.80±0.924	2.16±1.256	1.97±1.105

SD=standard deviations N=number of respondents

Table 2 - Cattle herd size and composition in TLU in smallholder farms.

Variables	Sululta (N=90) Mean±SD	TLU	Wolmera (N=90) Mean±SD	TLU	Overall (N=180) Mean±SD	TLU
Cattle	14.69±11.619	13.006	11.37±3.903	11.391	13.03±8.802	12.29
L.M. cows	1.68±0.837	1.68	1.83±0.993	1.83	1.76±0.920	1.76
C.M. cows	3.30±4.511	4.95	2.21±1.252	3.315	2.79±3.445	4.185
Calves	3.23±3.083	0.646	2.76±1.126	0.552	3.01±2.357	0.602
Heifers	2.78±3.131	1.668	2.16±1.094	1.296	2.47±2.359	1.482
Bulls	1.56±0.940	1.872	1.39±0.549	1.668	1.48±0.777	1.776
Oxen	2.19±0.518	2.19	2.73±1.166	2.73	2.48±0.956	2.48

SD =standard deviation; TLU= tropical livestock units; N= number of respondents; TLU=250kg of live weight of livestock; L.M. cows=local milking cows; C.M. cows= crossbred milking cows

Table 3 - Observed milking practices in the study areas (N=180)

Variable	Frequency		Percent	
	Yes	NO	Yes	Percent
Wash milkier hands and vessels	180	--	100.0	--
Wash udder before milking	129	51	71.7	28.3
Wash udder before and after milking	--	180	--	100.0
Use of individual towels	34	145	18.9	80.6
Use of collective towels	94	86	52.2	47.8
No towel	52	128	28.9	71.1

Table 4 - Average lactation length and daily milk yield of local and cross bred milking cow of small holder farmers.

Variables	Sululta(N=90) Mean ± S.D	Wolmera(N=90) Mean ± S.D	Overall(N=180) Mean ± S. D
Lactation length of local cattle in month	6.353± 0.7681	6.167 ± 0.5567	6.26 ± 0.6624
Lactation length of cross bred cows in month	9.72 ± 0.45	9.68 ± 0.47	9.7 ± 0.46
Average daily milk yield of local cows(/liter/day)	1.809± 0.4574	1.96± 0.8193	1.889 ± 0.6707
Average daily milk yield of cross bred cows(/lit/day)	9.56 ± 3.010	8.60 ± 2.703	9.11 ± 2.902

SD= standard deviations; N=number of respondents

Table 5 - Milk production and partition in to different use categories at smallholder farm level.

Category/area of study		Mean ± S. D	95% CI	Df	F ratios	P-value
Milk produced at farm/lit/day	Sululta	28.01±3.135	27.35-28.67	1	10.762	0.001**
	Wolmera	25.74±5.756	24.54-26.95	178		
	Overall	26.88±4.76	26.18-27.58	179		
Milk processed/lit/day	Sululta	1.29±1.493	0.98-1.6	1	0.215	0.643
	Wolmera	1.18±1.713	0.82-1.54	178		
	Overall	1.23±1.603	1.00-1.47	179		
Milk consumed at home/lit/day	Sululta	1.43±1.272	1.17-1.7	1	2.534	0.113
	Wolmera	1.16±1.059	0.93-1.38	178		
	Overall	1.29±1.176	1.12-1.47	179		
Milk sold/lit/day	Sululta	24.11±4.67	23.13-25.09	1	4.245	0.041*
	Wolmera	22.52±5.631	21.34-23.7	178		
	Overall	23.32±5.22	22.55-24.08	179		

**P-value is significant at 0.01 levels; SD= standard deviation; CI = confidence interval; df= degree of freedom

Milk marketing

Table 6 shows distance between production and market place. Nearly 54.9% of the households were nearby to the market center for their raw milk marketing while about 4% of the households travel more than 10 km.

Milk sales outlet

The main outlets for raw milk identified as shows in (Table 7) were cooperatives, processors, vendor, directly to consumer and hotels/restaurants 55.6%, 20.0%, 20.0%, 2.8% and 1.7% respectively.

Raw Milk marketing constraints

Table 8 illustrates raw milk marketing constraints at specific study area. The respondent farmers indicated that, price variations (87.2%), lack of fair market (72.2%) and lack of demand (49.4%) during fastening were the major problem of raw milk marketing in descending order of importance. As shows on table 9 milk price decided by producer, processor and collector were 6.1%, 25%, and 68.9% respectively as ascending order. Additionally through group discussion almost the entire group member pointed out they have less /no power to decided milk price at the study area.

Quality based payment was also another raw milk marketing constraints of the study area. They indicated quality based payment was enhanced quality of milk supplied to processors at the same time as encouraging them to produce more and quality milk. Through group discussions of respondents in the sturdy areas pointed out they possessed less preserving facilities for surplus milk produced and demand especially during fasting were great influence on raw milk marketing. Additionally, they showed that less adopted technologies for enhancing shelf life of raw milk in the study areas.

Table 6 - Distance of market center for milk in smallholder dairy farmer of study areas (N=175 households).

Distance of marketing place	Frequency	Percent
Less than 1 km (nearby)	96	54.9
Between 1 – 5 km (proximity)	70	40.0
Between 5 – 10 km (intermediate)	2	1.1
More than 10 km(far)	7	4.0

Table 7 - Marketing channel of smallholder farmer of the study area.

Milk out let	Frequency	Percent
Cooperatives	100	55.6
Hotels/restaurants	3	1.7
Vendor	36	20.0
Processors	36	20.0
Directly to Consumer,	5	2.8

Table 8 - Descriptions of marketing problems of small holder at the study area

Constraints	Frequency	Percent	Frequency	Percent
	Yes		NO	
Price variations	157	87.2	23	12.8
Lack of fair market	130	72.2	50	27.8
Lack of demand during fastening	89	49.4	91	50.6

Table 9 - Marketing channel of smallholder farmer of the study area.

Decisions of milk price	Frequency	Percent
Producer	11	6.1
Processor	45	25
Collector	124	68.9

Factors influencing milk production, consumption and marketing

Milk production was positively and significantly correlated with experience of raising cattle for milk productions, raw milk sold ($P<0.01$) and significantly correlated with distance of milk marketing ($P<0.05$). Milk sold was positively and significantly correlated with experience of raising cattle, milk productions and distance of milk sold ($P<0.01$). Milk consumption was negatively and significantly correlated with cattle herd size ($P<0.01$) (Table 10).

Physicochemical and microbiological quality of milk

The average chemical compositions of milk for fat (%), protein (%) and solids not fat (%) content were 3.6043 ± 0.12200 , 2.9749 ± 0.05147 and 6.9992 ± 0.13452 in raw milk samples mixture from producer respectively. Additionally, the mean of milk chemical compositions for fat (%), protein (%) and solids not fat (%) content were

3.3243±0.15814, 2.8929±0.08510 and 6.7114±0.24844 in raw milk samples mixture from collector respectively. The average physical properties of milk sample indicate on (Table 11) with density, freezing point 1.02721±0.000477 and -0.47143±0.00774 in raw milk sample from producer; 1.02623±0.000874 and -0.45788 ±0.016510 in raw milk sample from collector respectively. The overall Average of microbiological count of log (TBC cfu/ml), log (CC cfu/ml) and log (SCC/ml) of raw milk was 8.2577±0.10499, 3.3210±0.11295 and 5.0806±0.08484 for milk sample from the producer; 8.2577±0.10499, 3.3400±0.10352 and 5.1205±0.07533 for milk sample from collector respectively (Table 12). The overall mean of chemical compositions of milk for fat (%), protein (%) and solids not fat (%) contents were 3.5693±0.10892, 2.9646±0.04621 and 6.9632±0.12175 in raw milk sample mixture from the two districts respectively. Additionally the overall mean of physical properties of milk sample indicated on (Table 13) with density, freezing point 1.02665±0.00061 and -0.47088±0.0070 in raw milk sample from the two districts. The ANOVA showed significance difference (P<0.05) due to the source area of raw milk samples for fat, protein and freezing point. Moreover, ANOVA showed highly significant difference at (P<0.01) due to the source area of raw milk samples for solid not fat. The overall mean of the microbiological count of log (TBC cfu/ml), log (CC cfu/ml) and log (SCC/ml) of raw milk was 8.2285±0.10041, 3.3363±0.10010 and 5.1622±0.07382 for milk sample from the two districts respectively (Table 14). The ANOVA showed significant difference at (P<0.01) due to the source area for log (TBC cfu/ml)

Relationship among and between the physicochemical and microbiological test of Milk

Milk protein was positively and significantly correlated with fat, solid not fat and density of milk (P<0.01) and significantly correlated with each other (P<0.01). Milk protein was negatively and significantly correlated with added water and freezing point. Fat, solid not fat (SNF) and density of milk were also negatively and significantly correlated with added water and freezing point (P<0.01) (Table 15).

Table 10 - Correlations among different characteristics of small holder dairy farmers

Variable	Family size	ERCMP	Cattle	MPF/day	MS/day	MC/day	DMP
Family size	1						
ERCMP	0.149*	1					
Cattle	0.124	0.012	1				
MPF/day	0.121	0.375**	-0.119	1			
MS/day	0.085	0.342**	-0.146	0.908**	1		
MC/day	-0.006	-0.160	-0.613**	0.103	0.091	1	
DMP	-0.155*	0.232**	0.235	0.175*	0.284**	-0.080	1

*correlation is significant at the 0.05 level; **highly significant at the 0.01 level; ERCMP=experience of raising cattle for milk productions; MPF= milk produced at farm; MS= milk sold; MC= milk consumed and DMP= distance of marketing place.

Table 11 - Physicochemical properties of milk at farm and collection points in study area.

Variables and category	N	Mean ± Std. Error	95% CI	
Fat (%)	Producer	49	3.6043±0.12200	3.3590 - 3.8496
	Collector	7	3.3243±0.15814	2.9373 - 3.7112
	Overall	56	3.5693±0.10892	3.3510 - 3.7876
Protein (%)	Producer	49	2.9749±0.05147	2.8714 - 3.0784
	Collector	7	2.8929±0.08510	2.6846 - 3.1011
	Overall	56	2.9646±0.04621	2.8720 - 3.0572
SNF (%)	Producer	49	6.9992±0.13452	6.7287 - 7.2696
	Collector	7	6.7114±0.24844	6.1035 - 7.3193
	Overall	56	6.9632±0.12175	6.7192 - 7.2072
Density	Producer	49	1.02721±0.000477	1.02625 - 1.02817
	Collector	7	1.02623±0.000874	1.02410 - 1.02837
	Overall	56	1.02709±0.000432	1.02622 - 1.02795
Added water (%)	Producer	40	14.4087±1.45661	11.4625 - 17.3550
	Collector	7	14.2700±3.05519	6.7942 - 21.7458
	Overall	47	14.3881±1.30856	11.7541 - 17.0221
Freezing point	Producer	49	-0.47143±0.00774	-0.4870 - (-0.4559)
	Collector	7	-0.45788±0.016510	-0.4983 - (-0.4175)
	Overall	56	-0.46974±0.007066	-0.4839 - (-0.4556)

SE = Standard error; CI = confidence interval N= number of sample

Table 12 - Microbiological quality of milk at farm and collection points in study area.

Variables and category		N	Mean ± Std. Error	95% CI
Log (CC/cfu/ml)	Producer	49	3.3210±0.11295	3.0939 - 3.5480
	Collector	8	3.4569±0.26971	2.8191 - 4.0946
	Overall	57	3.3400±0.10352	3.1327 - 3.5474
Log (TBC/cfu/ml)	Producer	44	8.2572±0.11195	8.0314 - 8.4830
	Collector	8	8.2601±0.31391	7.5179 - 9.0024
	Overall	52	8.2577±0.10499	8.0469 - 8.4684
Log (SCC)	Producer	47	5.0806±0.08484	4.9098 - 5.2513
	Collector	8	5.3548±0.11766	5.0766 - 5.6331
	Overall	55	5.1205±0.07533	4.9694 - 5.2715

Log = logarithm in base ten (normal logarithm); CC = coli form count; SCC =somatic cell count; TBC = total bacterial count; cfu = colony forming unit per ml of milk sample; SE = standard error; CI = confidence interval

Table 13 - Physicochemical properties of milk for the two districts.

Variable		N	Mean ±Std. Error	95% CI	Df	F ratios	P-value
Fat (%)	Sululta	27	3.3185±0.13027	3.0507 - 3.5863	1	5.32	0.025*
	Wolmera	29	3.8028±0.16215	3.4706 - 4.1349	54		
	Overall	56	3.5693±0.10892	3.3510 - 3.7876	55		
Protein (%)	Sululta	27	2.8485±0.05218	2.7413 - 2.9558	1	6.46	0.014*
	Wolmera	29	3.0728±0.06985	2.9297 - 3.2158	54		
	Overall	56	2.9646±0.04621	2.8720 - 3.0572	55		
SNF (%)	Sululta	27	6.6307±0.14321	6.3364 - 6.9251	1	7.80	0.007**
	Wolmera	29	7.2728±0.17720	6.9098 - 7.6357	54		
	Overall	56	6.9632±0.12175	6.7192 - 7.2072	55		
Density	Sululta	27	1.02593±0.00051	1.02488 - 1.02698	1	1.27	0.266
	Wolmera	30	1.02730±0.00105	1.02514 - 1.02945	55		
	Overall	57	1.02665±0.00061	1.02544 - 1.02787	56		
Added water	Sululta	26	15.4562±1.80236	11.7441 - 19.1682	1	0.82	0.37
	Wolmera	21	13.0657±1.90496	9.0920 - 17.0394	45		
	Overall	47	14.3881±1.30856	11.7541 -17.0221	46		
Freezing point	Sululta	27	-0.45456±0.00987	-0.47485-(-0.43428)	1	5.21	0.026*
	Wolmera	30	-0.48557±0.00934	-0.50468 -(-0.46647)	55		
	Overall	57	-0.47088 ±0.0070	-0.48498- (-0.45679)	56		

** is highly significant at the (P< 0.01); * is significant at the (P< 0.05) level; SE = standard error; CI = confidence interval; Df = degree of freedom

Table 14 - Microbiological quality of milk for the two districts of study area

Variable		N	Mean±Std. Error	95% CI	Df	F	P
Log (CC/cfu/ml)	Sululta	30	3.3925±0.14411	3.0978 - 3.6873	1	0.302	0.585
	Wolmera	31	3.2819±0.14074	2.9944 - 3.5693	59		
	Overall	61	3.3363±0.10010	3.1361 - 3.5365	60		
Log (TBC/cfu/ml)	Sululta	27	7.9548±0.12902	7.6896 - 8.2200	1	7.774	0.007**
	Wolmera	29	8.4834±0.13799	8.2008 - 8.7661	54		
	Overall	56	8.2285±0.10041	8.0273- 8.4298	55		
Log (SCC)	Sululta	28	5.2643±0.10299	5.0530 - 5.4757	1	1.753	0.191
	Wolmera	31	5.0699±0.10406	4.8573 - 5.2824	57		
	Overall	59	5.1622±0.07382	5.0144 - 5.3099	58		

** is highly significant at the (P< 0.01); Log = logarithm in base ten (normal logarithm); CC = coli form count; SCC =somatic cell count; TBC = total bacterial count; cfu = colony forming unit per ml of milk sample; SE = standard error; CI = confidence interval

Table15 - Correlations among different characteristics of physicochemical and microbiological test of milk from selected area of small holder dairy farmers

Milk	Fat (%)	Prot. (%)	SNF	Density	AW	Fp	Log (CC)	Log (Tbc)	Log (SC)
Fat (%)	1								
Prot. (%)	0.671**	1							
SNF	0.624**	0.977**	1						
Density	0.565**	0.969**	0.997**	1					
AW	-0.562**	-0.951**	-0.984**	-0.976**	1				
Fp	-0.634**	-0.918**	-0.942**	-0.529**	1.000**	1			
Log (CC)	-0.148	-0.062	-0.068	-0.141	0.089	0.053	1		
Log (TBC)	0.138	0.075	0.070	0.030	-0.066	-0.081	-0.075	1	
Log (SCC)	0.068	0.075	0.074	0.001	-0.228	-0.127	-0.097	0.005	1

**= Correlation is significant at the ($P < 0.01$) level; SNF = solid not fat, AW = added water; Fp = freezing point; log (CC) = logarithms of Coli form count; log (TBC) = logarithms of total bacterial count; log (SCC) = logarithms of somatic cell count.

DISCUSSION

The overall mean family size obtained in the present study for all respondents was 5.69 ± 1.87 person's less than those reported by Tolera (2007) for Girar Jarso (5.77 persons) and by Abera (2008), 6.12 persons per house hold at kuyu district. The family size ranged from 2 to 12 persons at the study area which is comparable with the report of Kelay (2002) that family size ranged from 1 to 13 persons in Addis Ababa. About 78.9% of the households were basic educations and above in this study. This value is by far higher than the report of Sisay (2006) for Gondar area (38.5%). This finding indicates that, the education coverage between the study areas were different.

The cattle herd size of the study area was 12.27 TLU. The work of Abdinasir (2000) indicated that the cattle herd size at Bilalo and Lemmu areas are 8.57 TLU and 10.38 TLU respectively. In the present study area, the cattle herd was dominated by crossbreds that results in larger TLU cattle herd size as compared with Bilalo and Lemmu of Arsi area.

The present study also showed that milk production was positively and significantly correlated with experience of raising cattle for milk productions, raw milk sold ($P < 0.01$) and significantly correlated with distance of milk marketing ($P < 0.05$). Whereas the family sizes were not correlated with cattle herd size. On contrast finding reported by Abbinasir (2000) and Kelay (2002) indicated that family size and cattle herd size were positively and significantly correlated. These variations may be due to hired labor was means of overcoming family labor resource.

The average milk yield of cross bred cows in the study area was 9.11 ± 2.902 litres per day, which was comparable with average milk yield of 10 litres reported by Yoseph (1999), Azage et al. (2000), and Mekonnen et al. (2006). Moreover, the average milk yield of local cows was 1.889 ± 0.6707 which was comparable with reported by Zewdu (2004) indicated that the overall average daily milk yield of local cows in the first and second lactations in North Gonder Zone was 1.69 and 1.86 liters, respectively.

The overall average lactation length of local and crossbred cows was 6.26 ± 0.6624 and 9.7 ± 0.46 months, respectively in the study area. The lactation length of the indigenous cows observed in this study is comparable with the national average of 7 months (CSA, 2005). The lactation length in crossbred cows observed in this study is shorter than the lactation length of 11.7 months reported for crossbred cows in the central highlands of Ethiopia (Zelalem and Ledín, 2001a). The variation in lactation length in the present study may be credited to feed shortage and poor genetic potential of the sample population.

Overall mean of milk producing, processing, consuming and selling per day per household was 26.88 ± 4.76 , 1.23 ± 1.603 , 1.29 ± 1.176 and 23.32 ± 5.22 liters respectively. Eighty-six point seven percent (86.77%) of the milk produced in the area was sold by the producer through different channels. Amount of milk processed, consumed and used for calves was 4.6%, 4.8% and 3.84% respectively. This study is inconsistent with study conducted around Addis Ababa indicated that from total milk production 73% is sold, 10% is left for household consumption, 9.4% goes to calves and 7.6% is processed into butter (Azage and Alemu, 1998).

Marketing channels are routes through which products pass as they are moved from the farm to the consumer. From this study the main outlets for raw milk identified were cooperatives, processors, vendor, directly to consumer and hotels/restaurants. These are consistent with the result in any marketing system various actors participate in marketing of commodities and process of transactions made. These include itinerate /mobile traders, semi-whole sellers, retailers, cooperatives and consumers as reported by Holloway et al. (2000). Collectors collect the milk from the small holder and commercial dairy producers, they sale it to retailers, hotels, restaurants and processors. There

exist two types of collectors in the milk value chain. Cooperative collection centers are formal collectors organized by the bureau of agriculture in their respective districts. They have members of small holder dairy producers which supply daily production of milk in order to supply to the larger processors in Addis Ababa markets. In addition to collecting from cooperative and individual collectors, larger processors are also collect milk from smallholder farmers giving them additional cents over a liter of milk than other collectors. This condition had a negative effect on cooperative collection centers and mutual agreement and win-win approach should be followed among all the actors involving milk supply chain.

Among constraints of milk marketing, price variations, Lack of fair market and Lack of demand during fastening were the most indicated ones. The current study agreed with the report by [Baltenweck and Staal \(2000\)](#) for Kenyan highlands inaccessibility of fresh milk marketing. Through group discussion almost the entire group member pointed out they have less /no power to decided milk price at the study area. Quality based payment was also another raw milk marketing constraints of the study area. They indicated quality based payment was enhanced quality of milk supplied to processors at the same time as encouraging them to produce more and quality milk. Finally, milk marketing constraints were possessing less preserving facilities for surplus milk produced and demand especially during fasting were great influence on raw milk marketing.

Nearly 19% of the smallholders were using individual towels for cleaning udder of milking cows in 52.2% collective towels were used while in the rest (28.9%) no towel use practiced. It was reported by [Galton et al. \(1986\)](#) that pre-milking udder preparations play an important part in the contamination of milk during milking. Most of the dairy owners did not use towel and a few dairy owners used a single towel for all cows commonly to dry the udders. The reuse of towel for cleaning and sanitizing may result in recontamination of the udder. Since drying was not or insufficiently practiced, contamination level of milk was becoming higher.

The overall mean fat percentage (3.5693 ± 0.10892) of whole milk collected from the smallholder farmers in the current study is less than the fat content of whole milk collected from smallholder farmers reported by [Alganesh \(2002\)](#) for eastern Wollega (6.05%) and also slightly less than reported by [Asaminew \(2007\)](#) for Bahir Dar Zuria (4.14%). The variation in fat percentage observed in the present study may probably due to variation in stage of lactation, feeding regime and parity. The overall mean protein (2.9646 ± 0.04621) content from bulk milk obtained in the current study is lower than those reported by [O'Connor \(1994\)](#) for local cows' milk and also lower than [Zelalm and Ledin \(2001b\)](#) for whole milk in the central highlands of Ethiopia (3.1%). The average SNF (6.9632 ± 0.12175) content of milk obtained in the current study is slightly lower than reported by [Alganesh \(2002\)](#) for eastern Wollega (8.22%).

The overall mean total bacterial count of cows' milk produced in the study area was $8.2285 \log_{10} \text{cfu/ml}$. The total bacterial count obtained in this study is generally high as compared to the acceptable level of 1×10^5 bacteria per ml of raw milk ([O'Connor, 1994](#)). The current study is consistent with [Fekadu \(1994\)](#) reported that the minimum and maximum total bacterial count of raw cows' milk produced in southern region to be 6 to 8.8 $\log_{10} \text{cfu/ml}$. Commonly, lack of knowledge about clean milk production and use of unclean milking equipment would be some of the factors which contributed to the poor hygienic quality of milk produced in the study area.

The overall mean coliform count of milk produced in the area was $3.3363 \log_{10} \text{cfu/ml}$. The coliform count of cows' milk obtained in the current study is smaller than reported given by [Fekadu \(1994\)](#) for districts of southern region ($3.8 \log_{10} \text{cfu/ml}$). The current result is also inconsistent with the reported by [Zelalem and Bernard \(2006\)](#) for cows' milk collected from different producers in the central highland of Ethiopia ($6.57 \log_{10} \text{cfu/ml}$). The higher coliform count obtained in this study may be due to the initial contamination of the milk samples either from the cows, the milkers, milk containers and the milking environment. The overall mean of somatic cell count in \log (SCC/ml) of raw milk was 5.1622 ± 0.07382 for milk sample from the two districts.

CONCLUSIONS AND RECOMMENDATIONS

Dairy production became a crucial element of the farming activities and income generating for household in "Sululta" and "Wolmera" district of Oromia special zone surrounding Addis Ababa.

The proportion of raw milk used for household consumption was relatively small and the major part of milk produced by smallholders is destined to market. Smallholders also process milk to butter and cheese. Milk was soured for 2-3 days before processing it in to butter and cheese. The main outlets for raw milk identified were cooperatives, processors, vendor, directly to consumer and Hotels/restaurants. Price variations, lack of fair market, lack of demand during fastening, lack of quality based payment and lack of preserving facilities were the major problem of raw milk marketing in the study areas.

Hygienic conditions of milking and storage processes, transferring of milk into different containers and sieves, unclean milk equipment were basic determinants of milk quality. Majority of raw milk samples from producer and collector bulk milk sample had higher TAPC and coliform counts, which was higher than the international acceptable limits.

Based on the aforementioned conclusions, the following recommendations are forwarded:

- Smallholders should be provided/supported with extension and training opportunities for hygienic conditions of milking, storing and processing;
- Quality based pricing in the milk value chain could contribute as incentive to producers for production of quality mil;
- Smallholders should be provided/supported with credit facility and market information;
- Regulatory mechanisms should be established and enforced to deter milk and milk products adulteration.

DECLARATIONS

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Author's contribution

Both authors have contributed equivalent effort for this manuscript.

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Competing interests

The authors have not declared any conflict of interests.

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IMPACT OF BODY CONDITION, MILK YIELD AND BLOOD METABOLITE CONCENTRATIONS ON THE RESUMPTION OF OVARIAN ACTIVITY IN CATTLE

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[✉]Supporting Information

ABSTRACT: Body condition of cows, level of milk production and concentrations of blood metabolites can influence the resumption of ovarian activity in cattle. This study investigated the relationships among body weight (BW), body condition score (BCS), milk yield, blood metabolite [Glucose, total protein, albumin, globulin, urea, triglyceride, non-esterified fatty acids (NEFA) and Beta-hydroxybutyrate (BHB)] concentrations and resumption of postpartum ovarian cyclicity in 20 Sanga and 20 Friesian × Sanga cows. Cows were classified as having resumed ovarian activity when they had plasma progesterone concentration of ≥ 1 ng/mL. Based on the resumption of ovarian activity, cows were also classified into three ovarian cyclicity groups namely; early resumption of ovarian cyclicity (≤ 56 days postpartum), late resumption of ovarian cyclicity (57-112 days postpartum) and non-resumption of ovarian cyclicity (by 112 days postpartum). BW, BCS and milk yield did not affect ($P < 0.05$) cycling status in Sanga cows. Friesian × Sanga that resumed ovarian cyclicity early (2.54 L/day) or late (2.01 L/day) had higher milk yield ($P < 0.05$) than their counterparts that did not resume ovarian cyclicity during the period of study (1.61 L/day). However, BW and BCS were similar ($P > 0.05$) in the three ovarian cyclicity groups in the Friesian × Sanga cows. Sanga cows that resumed ovarian cyclicity early had lower total protein concentration than those that resumed ovarian cyclicity late (76.8 versus 89.3 g/L; $P < 0.05$). Globulin concentration was also lower ($P < 0.05$) in cows that resumed cyclicity early (42.6 g/L) than late (54.2 g/L) and non-cycling (49.7g/L) Sanga cows. There were significant ($P < 0.05$) positive and negative correlations among some of the plasma metabolites determined. In conclusion, earlier resumption of ovarian cyclicity was associated with higher milk yield in Friesian × Sanga cows and lower concentrations of total protein and globulin in Sanga cows. Improved feeding and management strategies are recommended for the achievement of reproductive success in cows.

Keywords: Body Weight, Cow, Nutritional Status, Ovulation, Relationships, Supplement

INTRODUCTION

The changes in health, nutritional and physiological status of most cows during the transition period adversely affects their performance. The period of late pregnancy is marked by increased growth and development of the foetus which consequently increase the nutritional needs of pregnant cows. Additionally, increased dietary intake fails to keep pace with rising milk production during early lactation leading to a state of negative energy balance (Esposito et al., 2014). Cows mobilize their lipid reserves during this period as an additional source of energy for maintenance and milk production resulting in body condition loss and increase in blood concentrations of non-esterified fatty acids (NEFA) and ketone bodies such as beta-hydroxybutyrate (BHB) (Denicke, 2018). Negative energy balance (NEB) affect reproductive performance adversely by inhibiting the release of luteinizing hormone, oestrogen and progesterone and the growth and development of ovarian follicles (Butler, 2003). Moreover, lower glucose, insulin and insulin-like growth factor I (IGF-I) concentrations during NEB in early lactation could delay ovulation in cows (Konigsson et al., 2008; Soca et al., 2014).

Cows in the extensive system of production in Ghana, depending mostly on natural herbage without feed supplementation face declining performance in terms of growth, milk production and reproductive performance as a consequence of seasonal variation in pasture availability and important nutrients such as energy and protein (Obese et al., 2018). Feeding and management is important for metabolic adaptation and achievement of reproductive success in cows during the transition period (Drackley and Cardoso, 2014; Lucy et al., 2014). There is, however, a dearth of information on relationships among body weight, body condition score, concentrations of blood metabolites and resumption of postpartum ovarian activity in dual-purpose cows in the extensive system of cattle production in the Accra plains of Ghana.

This study therefore, evaluated the relationships among body weight (BW), body condition score (BCS), milk yield, blood metabolite concentrations and resumption of postpartum ovarian activity in Sanga and Friesian × Sanga

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crossbred cows provided feed supplement in the extensive system of cattle production in the Accra plains of Ghana. The associations among plasma concentrations of blood metabolites were also investigated.

MATERIALS AND METHODS

Location of study

The study was conducted at the Animal Research Institute's Katamanso Station located in the Accra Plains on latitude 05° 44' N and longitude 00° 08' W. The vegetation is grassland with sparsely distributed shrubs. The area has a bimodal rainfall pattern with the major wet season occurring from April to July and a minor season from September to November. The remaining months constitute the dry period. Annual rainfall and temperatures range between 600-1000 mm and 21°C to 33°C respectively and relative humidity ranges from 69 to 94% (Obese et al., 2015). The study received approval from the In-house Committee for Research of the Animal Research Institute.

Management of animals

Forty multiparous made up of 20 Sanga and 20 Friesian × Sanga cows in their second to fifth lactation were used in a study which lasted for 16 weeks during the postpartum period. They calved between January and February (mostly in the dry season). At the start of the experiment, the Sanga cows (n=20) had a mean (\pm SEM), BW of 289.6 \pm 4.9 kg and BCS (BCS) of 7.1 \pm 0.28 (scale 1 – 9; Nicholson and Butterworth, 1986). The Friesian × Sanga cows (n=20) had an average BW of 291.2 \pm 9.8 and BCS of 6.9 \pm 0.26. The two herds were housed separately in open kraals and also grazed separately, but on plots within the same field of natural pasture with similar nutritive value. The natural pasture grazed comprised a mixture of grasses and broad-leaved plants such as *Panicum insularis*, *Sporobolus pyramidalis*, *Brachiaria deflexa*, *Milletia thonningii*, *Griffonia simplicifolia*, *Grewia carpinifolia*, *Stylosanthes hamata* and *Stylosanthes guineensis*. Grazing period was from 08.00 h to 16.00 h daily. Cows received a supplementary diet (Table 1) before grazing. The supplement had a crude protein of 16% and digestible energy of 13.9 MJ/Kg DM. Each cow received 2.5 kg of the supplement for a period of 16 weeks. Water was provided in morning and evening. Cows were milked once daily in the morning between 05.00 h to 06.30 h.

Partial milking was practiced with milk collected from two quarters of the udder, and the other two quarters were reserved for the calves. Mating was natural with service bulls running freely with females all year round. Calves were weaned at about six months of age. Cows and their calves were treated against ecto-parasites, and endo-parasites once a month during the dry season and fortnightly in the wet season. They were also treated against diseases as the need arose and vaccinated against contagious bovine pleuropneumonia once a year as has been reported in earlier studies (Obese et al., 2018). Cows were weighed weekly and the BCS of cows determined weekly using a 9-point score (1= very thin to 9 = obese; Nicholson and Butterworth, 1986).

Blood sampling

Blood samples were collected from cows once every week, from week 1 to 16 postpartum after morning milking at 06.30 h by jugular venipuncture into a 7.5-mL EDTA-coated vacutainer tubes (BD Vacutainer Systems, Plymouth, UK) for analysis of metabolic hormone (progesterone) and blood nutritional metabolites (total protein, albumin, triglyceride, and urea, NEFA and BHB). Blood samples for determining concentrations of glucose was collected into evacuated tubes containing fluoride oxalate. All samples collected were then placed on ice immediately after collection and transported to the laboratory where plasma was separated by centrifugation at 1800 × g for 15 min at 4°C. The plasma samples were stored at -20°C, until assayed for the blood metabolites.

Blood metabolite analyses

The concentrations of glucose, total protein, albumin, triglyceride and urea were determined in the plasma at weeks 1, 3, 5, 7, 9, 11, 13 and 15 using the Mindray BA-88A Semi-Auto Chemistry Analyzer (Nanshan, China). Globulin concentration was computed as the difference between the total protein and albumin concentrations. The concentration of NEFA in the plasma was determined by enzymatic calorimetric techniques using an assay kit (Diasys Diagnostic Systems, Germany) while plasma BHB concentration was measured using a BHB assay kit (Randox Laboratories, UK). The BHB and NEFA concentrations in the plasma were determined at weeks 1, 3, 5, 7 and 9.

Resumption of postpartum ovarian activity and conception were determined by measuring the progesterone concentrations in plasma samples from cows from week 1 to week 16 postpartum (the end of the study period). Plasma progesterone concentrations were determined using a commercial ELISA Kit (DiaMetra, S.r.l, Italy). Cows were classified as having resumed ovarian activity when plasma progesterone concentration of \geq 1 ng/mL was recorded in plasma samples (Tamadon et al., 2011). Cows were classified as not cycling if progesterone concentration remained below 1 ng/mL throughout the study period. Based on the resumption of ovarian activity, cows were also classified into three ovarian cyclicity groups namely; early resumption of ovarian cyclicity (\leq 56 days postpartum), late

resumption of ovarian cyclicity (57-112 days postpartum) and non-resumption of ovarian cyclicity (>112 days postpartum). The progesterone assay had a sensitivity of 0.05 ng/mL.

Statistical analyses

The effects of BW, BCS, milk yield and plasma concentrations of metabolites (glucose, total protein, albumin, globulin, triglyceride, urea, NEFA and BHB) on resumption of ovarian activity in Sanga and Friesian × Sanga cows were analysed using repeated measures analysis of variance procedure of GenStat Release 12th Edition (GenStat, 2009). Pearson’s partial correlation coefficients were calculated to describe linear relationships among the concentrations of plasma metabolites using the IBM SPSS v.22.0 (2013). Values reported are least square means and SEM, unless otherwise stated. Mean values were considered to be statistically significantly different when $P < 0.05$ and considered a tendency when $P < 0.10$ but > 0.05 .

Table 1 - Composition of supplement (concentrate)

Ingredient	Composition (%)
Maize	40.0
Wheat Bran	42.0
Soya bean Meal	10.0
Dicalcium Phosphate	2.0
Oyster Shell meal	5.0
Salt	0.5
Premix*	0.5
TOTAL	100

* The premix provided the following per kg of concentrate: Vit A= 30,000 IU, Vit D = 35,000 IU, Vit K = 33.75 mg, Vit B = 210 mg, Se = 0.375 mg, Mn = 150 mg, Iodate 5 = mg, Zn = 125 mg, Cu = 15 mg, Choline Chloride = 300 mg and Antioxidant 62.5 mg.

RESULTS AND DISCUSSION

Body condition score (BCS) and milk yield

The effect of BW, BCS and partial milk yield on the resumption of ovarian cyclicity in the two breeds of cows are presented in Table 2. BCS at calving or loss of BCS during the early postpartum period could inhibit resumption of ovarian cycles and increase calving to conception intervals in cows (Roche et al., 2009; Soca et al., 2014). In the present study, BW or BCS did not affect ($P > 0.05$) cycling status in Sanga or Friesian × Sanga cows. The overall mean BCS of range of 7.18 - 7.25 for the Sanga and 6.73 - 7.06 for the Friesian × Sanga indicates there were in moderate to good condition on the scale of 1-9 (1=thin, 9= obese; Nicholson and Butterworth, 1986). Higher nutrient demands for milk production especially during early lactation could induce negative energy balance in cows leading to delays in ovulation and abnormal resumption of ovarian cycles (Esposito et al., 2014). Friesian × Sanga cows that resumed ovarian activity early were probably able to meet their nutrient requirements and partitioned it into milk production accounting for their higher ($P < 0.05$) milk yield than their counterparts that did not resume ovulation (2.54 versus 1.61 L/day; Figure 1). Also, nutritional status is known to influence the secretion of hormones such as growth hormone involved in milk production and other metabolic hormones including luteinizing hormone, insulin and insulin-like growth factor-I which are associated with resumption of ovarian activity in cows (Diskin et al., 2003; Peter et al., 2009). The results in the present study is at variance with the report of Obese et al. (2015) who observed no significant differences in milk yield among early, late or non-cycling Friesian × Sanga cows which grazed extensively on natural pasture without any feed supplementation. Variation in the nutritional status of cows may account for the observed differences.

Table 2 – Effect of body weight, body condition score and partial milk yield on resumption of ovarian cyclicity in Sanga and Friesian × Sanga cows during the postpartum period

Parameter	Sanga			SEM	P-value	Friesian x Sanga			SEM	P-value
	ERO (n= 6)	LRO (n=5)	NRO (n=9)			ERO (n=7)	LRO (n=7)	NRO (n=)		
BW (kg)	273.42	294.30	289.4	9.92	0.269	288.1	298.2	296.8	15.54	0.868
BCS	7.18	7.25	7.18	0.036	0.973	7.06	6.73	6.73	0.043	0.642
Milk yield (L/day)	1.69	1.67	1.53	0.022	0.659	2.54 ^a	2.01 ^{ab}	1.61 ^b	0.039	0.011

n = number of cows; ERO= Early resumption of ovarian activity (≤ 56 days); LRO=Late resumption of ovarian activity (57-112 days); Non-resumption of ovarian activity (by 112 days); BCS = Body condition score; Means in the same row within each breed with different superscripts (a, b) are significantly different ($P < 0.05$)

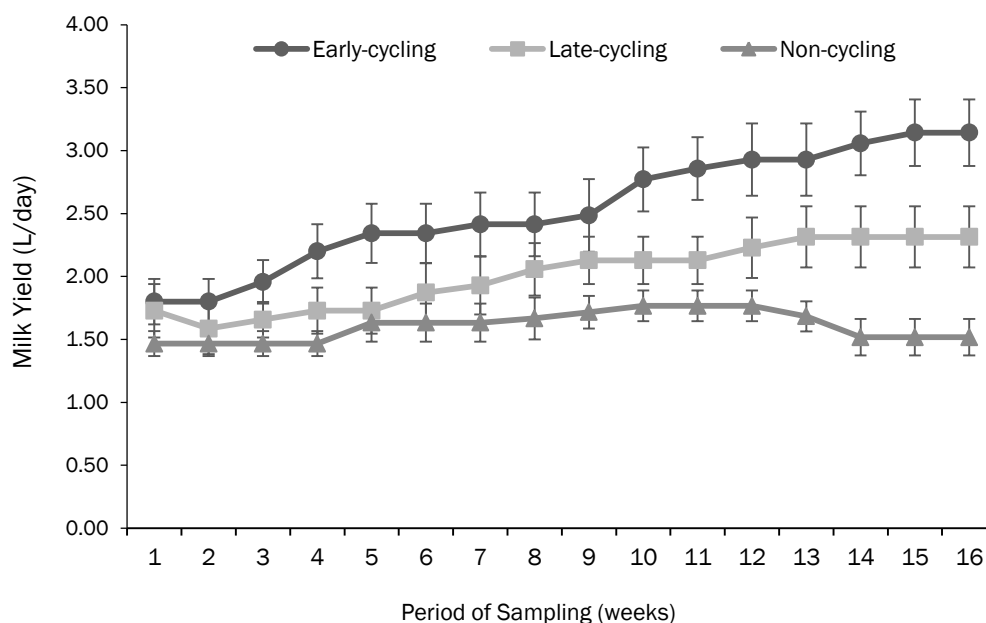


Figure 1 - Partial Milk yield in Sanga and Friesian × cows with early, late or non-resumption of ovarian cyclicity during the postpartum period.

Blood metabolite concentrations

The blood metabolite concentrations in cows that resumed ovarian cyclicity early, late or failed to resume ovarian cyclicity by 112 days postpartum is shown in Table 3. Blood glucose, albumin, triglyceride, urea, NEFA and BHB concentrations were similar ($P>0.05$) in Sanga cows suggesting no relationship of these metabolites with the resumption of ovarian activity. Also, the non-significant differences ($P>0.05$) in the concentrations of all plasma metabolites determined in the Friesian × Sanga cows indicates no correlation of these metabolites with resumption of ovulation in this breed.

Glucose is a major blood metabolite defining the energy metabolism in cattle. The overall mean glucose concentrations in the two breeds fell within the normal physiological range of 2.2–5.6 mmol/L for cattle ([The Merck Veterinary Manual, 2010](#)) suggesting adequate energy supply to the cows. Sanga cows resumed ovarian cyclicity early had lower total protein concentrations in the blood than those that cycled late (76.8 versus 89.3 g/L; $P<0.05$). [Ahmad et al. \(2004\)](#) also reported of lower total protein concentrations in cycling than non-cycling cows. However, higher concentrations of total protein in cycling than non-cycling cows have been reported in other studies ([EL-Azab et al., 1993](#); [Saleh et al., 2011](#)). The variations could be due to differences in breed and level of nutrition. Excessive intake of protein in the diet coupled with inadequate supply of energy can delay resumption of ovarian activity and affect conception in cows by increasing urea nitrogen concentrations in the blood thus interfering with uterine function ([Bisinotto et al., 2012](#); [Bindari et al., 2013](#)). The overall mean total protein concentration in Sanga (83.1 g/L) and Friesian × Sanga (84.3 g/L) were within the normal range of 67 - 85 g/L reported for cows ([Otto et al., 2000](#); [Merck Veterinary Manual, 2010](#)). This suggests both breeds of cows were in good protein status. The overall mean albumin concentrations obtained in the present study were 34.2 g/L and 33.4 g/L for the Sanga and Friesian × Sanga breed respectively. These were within the normal range of 25.0–38.0 g/L reported for cows ([The Merck Veterinary Manual 2010](#)) and suggest that the cows used in this study were not malnourished since albumin concentration in the blood significantly correlate with nutritional status in cattle ([Coppo, 2004](#)).

Circulating concentrations of globulin usually give an indication of an animal's immune state and its response to fighting diseases and infections ([Kapale et al., 2008](#)). The significantly ($P<0.05$) lower globulin concentration in early (42.6 g/L) than late (54.2 g/L) and non-cycling (49.7 g/L) cows ([Table 3](#)) is contrary to the expectation that cows with lower globulin concentrations should rather experience delayed resumption of ovarian activity. Cows with periparturient diseases (endometritis or mastitis) have been found to have delayed resumption of ovarian activity and conception ([LeBlanc et al., 2002](#); [Williams et al., 2007](#)). However, cows in this study were not physically observed to have mastitis or endometritis. Lipid metabolism parameters include blood triglyceride and total cholesterol levels. The overall mean triglyceride concentrations of 0.27 and 0.25 for Sanga and Friesian × Sanga cows were within the normal physiological range of 0.1 - 0.3 mmol/L and suggested the preserved liver function within fat metabolism ([Şamanc, 2009](#)).

Urea levels in the blood serve as indicator of protein utilization and the similar concentrations in the cycling and non-cycling groups in both breeds could imply efficient utilization of protein in both breeds of cows. The overall mean values of 5.30 and 5.05 mmol/L obtained for the Sanga and Friesian × Sanga cows were within the normal physiological range of 3.6-8.9 reported for cows (The Merck Veterinary Manual, 2010). Blood levels of NEFA and BHB are indicators for energy metabolism. Moreover, higher NEFA concentrations have been associated with anovulatory postpartum anoestrus in cows (Butler, 2003, Montagner et al., 2016). However, blood NEFA concentrations in the two breeds were not associated with resumption of ovarian cyclicity in the present study.

Table 3 – Plasma concentrations of metabolites in Sanga and Friesian × Sanga cows with early, late or non-resumption of ovarian cyclicity during the postpartum period.

Parameter	Sanga			SEM	Overall Mean	P-value	Friesian x Sanga			SEM	Overall Mean	P-value
	ERO (n=6)	LRO (n=5)	NRO (n = 9)				ERO (n=7)	LRO (n=7)	NRO (n=6)			
Glucose (mmol/L)	4.42	4.44	4.42	0.082	4.43	0.989	3.97	4.14	4.16	0.065	4.09	0.506
Total Protein (g/dL)	76.8 ^b	89.3 ^a	83.1 ^{ab}	2.60	83.1	0.024	83.0	86.0	83.9	1.73	84.3	0.722
Albumin (g/L)	34.2	35.0	33.5	0.67	34.2	0.505	32.9	34.2	33.0	0.607	33.4	0.492
Globulin (g/L)	42.6 ^b	54.2 ^a	49.7 ^a	2.16	48.8	0.018	50.2	51.6	50.8	1.67	50.9	0.962
Triglyceride (mmol/L)	0.26	0.27	0.27	0.009	0.27	0.326	0.24	0.25	0.26	0.925	0.25	0.289
Urea (mmol/L)	5.18	4.96	5.76	0.148	5.30	0.563	5.10	4.83	5.21	0.081	5.05	0.226
BHB (mmol/L)	0.29	0.38	0.38	0.020	0.35	0.226	0.40	0.46	0.37	0.021	0.41	0.340
NEFA (mmol/L)	0.17	0.17	0.20	0.010	0.18	0.447	0.17	0.18	0.21	0.011	0.19	0.655

N= number of cows; ERO= Early resumption of ovarian activity (≤56 days), LRO=Late resumption of ovarian activity (57-112 days), Non-resumption of ovarian activity (by 112 days); Means in the same row within each breed with different superscripts (a, b) are significantly different (P<0.05)

Correlation among blood metabolites

The partial correlation coefficients among concentrations of the plasma metabolites, glucose, total protein, albumin, globulin, triglycerides, urea, BHB, NEFA and urea are presented in Table 4. There were negative and significant relationships between glucose and total protein ($r = -0.255$; $P < 0.01$), globulin ($r = -0.200$; $P < 0.01$) and triglyceride ($r = -0.2550$; $P < 0.01$) suggesting these metabolites are affected in the opposite direction in the nutritional management of the cows during the postpartum period. Total protein was significant and positively correlated with albumin ($r = 0.371$; $P < 0.01$), globulin ($r = 0.763$; $P < 0.01$) and triglyceride ($r = 0.485$; $P < 0.01$) an indication that variations in plasma total protein concentration invariably influence plasma concentrations of albumin, globulin and triglyceride during the postpartum period. Obese et al. (2015b) in an earlier study observed a significant positive relationship between plasma total protein and globulin concentration in Friesian × Sanga cows grazing extensively on natural pasture without feed supplementation in the Accra plains of Ghana. On the other hand significant and negatively correlation between albumin and globulin ($r = -0.317$; $P < 0.01$) suggest the opposite influence of nutritional management on the concentrations of these metabolites. The significant and positive relationships between albumin and triglyceride ($r = 0.473$; $P < 0.01$), albumin and BHB ($r = 0.218$; $P < 0.01$), albumin and NEFA ($r = 0.183$; $P < 0.05$) may indicate similar influence of nutritional management on the concentrations of these metabolites during the period of study. This also applies to the relationship between globulin and triglyceride ($r = 0.166$; $P < 0.01$). However, triglyceride concentration was significant and negatively correlated with urea ($r = -0.119$; $P < 0.05$) and NEFA ($r = -0.188$; $P < 0.05$) suggesting opposite influence of nutritional management on these two metabolites.

Table 4 – Partial correlation coefficients among plasma concentrations of glucose, total protein, albumin, globulin, triglycerides, urea, BHB and NEFA in Sanga and Friesian × Sanga Cows (n=40) during weeks 1,3,5,7, 9, 11, 13 and 15 postpartum

Variables	TP	Albumin	Globulin	Trig.	Urea	BHB	NEFA
Glucose	-0.255**	-0.088	-0.200**	-0.225**	0.034	0.079	0.014
TP		0.371**	0.763**	0.485**	-0.075	-0.042	-0.101
Albumin			-0.317**	0.473**	-0.044	0.218**	0.183*
Globulin				0.166**	-0.046	-0.098	-0.142
Trig.					-0.119*	-0.085	-0.188*
Urea						0.029	0.021
BHB							0.096

TP = Total Protein; Trig = Triglycerides; BHB = Betahydrobutyrate; NEFA = Non-esterified fatty acid; *Significant at $P < 0.05$; **Significant at $P < 0.01$; Correlation among all metabolites except NEFA and BHB = 306; Correlation among NEFA, BHB and other metabolites = 196

CONCLUSION

Earlier resumption of ovarian activity was associated with higher milk yield in Friesian × Sanga cows and lower concentrations of total protein and globulin in Sanga cows. Positive or negative relationships were observed among the concentrations of some of the metabolites measured. Improved feeding and management practices are recommended for the achievement of reproductive success in cows. Further studies should be carried out to evaluate the quality of feed supplements and the time of initiation of supplementation in such extensive systems of cattle production.

DECLARATIONS

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Authors' contribution

FYO designed the trial, supervised the data collection and laboratory work and contributed to the interpretation of the results and drafting of manuscript. LKA participated in the design of the study, interpretation of data and drafting of manuscript. KD contributed to data collection, laboratory and Statistical analysis.

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Competing interest

The authors declare that they have no competing interests.

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DIAGNOSIS OF SUBCLINICAL ENDOMETRITIS DURING POSTPARTUM PERIOD ON SUBSEQUENT PREGNANCY IN SMALL, MEDIUM AND LARGE SCALE DAIRY FARMS IN AND AROUND GONDAR, ETHIOPIA

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✉ Supporting Information

ABSTRACT: During the study period 147 apparently healthy 3rd trimester pregnant cows were selected in smallholder, medium and large scale dairy farms in and around Gondar, North Western Ethiopia from January 2015 to September 2017. After calving, endometrial samples were collected from the uterus of apparently normal cows by using uterine lavage technique on postpartum dairy cows from 40-60 days. Collected samples were centrifuged and a drop of sediment was streaked onto a clean microscopic slide and stained with giemsa. The percentage of polymorphonuclear cells (neutrophils) was calculated. It was found that increase in the number of neutrophils correlated with decrease in pregnancy. In conclusion, subclinical endometritis diagnosed by endometrial cytology was associated with reduced rate of pregnancy.

Keywords: Subclinical Endometritis, Endometrial Cytology, Pregnancy, Neutrophils

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INTRODUCTION

Subclinical endometritis is a chronic, unapparent inflammatory process of endometrium with a relatively high proportion of polymorphonuclear cells (Neutrophils) cells in the uterus, which suppresses the fertility of affected cows. Proportion of polymorphonuclear cells (Neutrophils) cells considered to be “relatively high” depends on sampling technique as well as on the time from parturition (Sheldon et al., 2006). The key for excellent fertility in dairy herds is a healthy uterine environment, optimal estrus detection efficiency and ideal timing for breeding (LeBlanc et al., 2002). Subclinical endometritis in dairy cows and has a profound negative impact on pregnancy (Hammon et al., 2006).

Recent studies have focused on a more sophisticated diagnosis of endometrial alterations beyond clinical signs of endometritis. New techniques have been described for the diagnosis of subclinical endometritis (SE). The inflammation of the endometrium is characterized by the proportion of polymorph nuclear cells (PMN) in a cytological sample taken from clinically healthy cows. Cytological samples can be obtained by flushing the uterine lumen or by using the cytobrush technique (Sheldon et al., 2006). This latter technique uses a small brush that is inserted into the uterus to collect endometrial cells and to determine the proportions of PMN in the sample. Studies on SE found a prevalence of SE in the range between 12 % and 94 %. Different study designs and inconsistent definitions for SE hinder a valid comparison of the results of these studies. The time of examination in these studies varied from 21 to 60 day of postpartum (Gilbert et al., 2005).

Subclinical endometritis causes considerable infertility problems in the presence of uterine bacterial contaminations of disrupt the delicate hormonal milieu of the hypothalamic-pituitary ovarian axis and disrupt follicular growth and development of uterine infections have been reported to be associated with an increased incidence of cystic ovarian disease (Andrew et al., 2006).

Postpartum endometritis in cattle is a multifactorial disease with high economic impact. Inflammation of the bovine uterus has been demonstrated to decrease fertility. Both clinical and subclinical endometritis were associated with increased days to first service as well as decreased conception and pregnancy rates resulting in an increased risk of culling (Perea et al., 2005). The objective of this study was to diagnose subclinical endometritis and its effects on pregnancy in dairy cows.

MATERIALS AND METHODS

Study area

The study was conducted in urban and peri urban areas of Gondar town dairy farms which are located North West part of Ethiopia in Amhara regional state. Gondar town is found about 727 km from the capital city Addis Ababa. It is located at latitude, longitude, altitude of 12.3-13.8°N, 35.3-35.7°E and 2200 m.s.l, respectively. The annual mean minimum and maximum temperature of the area vary between 12-17°C and 22-30 °C, respectively. The area is located under woynadega, agro-climatic zone and receives a bimodal rainfall the average annual precipitation rate being 1000 mm that comes from the long and short rainy seasons. The short rainy season occur during the months of March, April and May while the long ones extend from June through September (CSA, 2008).

Study farms

The dairy farms considered for this study were categorized into defined strata based on cow herd size; these were small scale dairy farm (SSDF), medium scale dairy farm (MSDF) and large scale dairy farm (LSDF) having 1 or 2, 3 to 10 and 11 to above as described by ILRI (1996), respectively. During the study period 147 apparently healthy 3rd trimester pregnant cows were selected.

Study design

Cows were selected in smallholder, medium and large scale dairy farms and the study was conducted from January 2012 to September 2013. The owners of the farms were informed about the relevant characteristics of the study and agreed with the design. The dairy farms were visited every 15 days. Enrolment of cows, clinical examination and evaluation were performed by the same investigator. The cows were examined by using lavage between 40 and 60 days postpartum for the presence of sub clinical endometritis. Endometrial cells were collected by uterine lavage technique.

Clinical examination

In each cow a clinical examination of the reproductive tract was performed by vaginal examination and transrectal palpation of the uterus and the ovaries. Cows with vaginal discharge were diagnosed as affected by clinical endometritis and excluded from the study. In addition, cows which had received systemic or intrauterine antibiotic therapy within 6 days prior to enrollment were not selected for the study. Pregnancy diagnosis was performed by transrectal palpation of the uterus and its contents post insemination.

Cytological samples

The cows were examined between 40 and 60 days after calving for the presence of subclinical endometritis by using the lavage technique. Collected samples were centrifuged and a drop of sediment was streaked onto a clean microscopic slide and stained with Giemsa.

Subclinical endometritis was determined using endometrial cytology (Gilbert et al., 2005; Kasimanicham et al., 2005). To minimize contamination of the sample, the vulva and perineum were cleaned with water and soap properly. The uterus was lavaged by infusing 50 ml of 0.9% sterile sodium chloride solution with 50 ml syringe attached to a 52 cm sterile plastic infusion rod. The uterus was then manipulated and massaged through rectum for about 10 seconds, and some of the infused fluid was aspirated into the syringe via the same sterile plastic infusion rod by negative pressure aspiration and retracted to recover the fluid. No special effort was made to retrieve the fluid if it did not flow freely.

As much fluid as possible was recovered by negative pressure aspiration into the syringe and transferred to the 10 ml sterile test tube without any preservative. The uterine samples were put into the icebox and brought to the Faculty of Veterinary Medicine, Microbiology laboratory within 2 hours of collection and centrifuged at 800 rpm for 5 min. A drop of sediment was streaked on to a clean microscope slide and air-dried. Then the slide was fixed with methanol and stained with Geimsa for 45 min and examined under a microscope at 400× magnification. Initially the whole slide was assessed and a representative area was selected to determine the PMN % among all other cells was estimated. The percentage of neutrophils PMN % was determined by counting 80–100 cells on a representative field of vision. The threshold value for the proportion of PMN indicated samples with $\geq 3\%$ neutrophils were categorized as subclinical endometritis and cows were characterized as suffering from subclinical endometritis. The counted cells contained epithelial cells, neutrophils, large mononuclear cells (Presumed to be macrophages) and small mononuclear cells (Presumed to be lymphocytes). The samples that did not contain epithelial cells were considered not taken from uterus and rejected for the study.

Data management and statistical methods

To measure the impact of subclinical endometritis on subsequent pregnancy descriptive statistics for the amount of neutrophils were used. The data was analyzed using statistical package for social science (SPSS) (Version 18). The Generalized Linear Model was utilized to analysis the effect of selected factors on the amount of neutrophils. Multiple logistic regression and Kaplan-Meier survival analysis were applied to analysis the relationship between the amount of neutrophils and the conception rate in the first insemination after sampling. The Pearson correlation test and the Chi-square correlation test were used to analysis the impact of quantitative factors on each other and the impact of qualitative factors on each other respectively.

The student T test was used to analysis the impact of quantitative factors on qualitative factors and vice versa. A probability of $P < 0.05$ was set as the significance level. The receiver operating characteristic (ROC) analysis was applied to determine the most appropriate cutoff point for percentage of neutrophils in samples.

RESULTS

The incidence of subclinical endometritis was different in small, medium and large scale farms 25 (37.88%), 29(43.94%) and 13 (18.18%), respectively.

Only 72 (48.98%) of the selected cows became pregnant in AI after sampling, and 75 (51.02%) did not. The amount of neutrophils was lower in the cows that became pregnant in the first AI after sampling. With an increase in the number of neutrophils the likelihood of pregnancy decreased. The mean number of services per conception as 2.04 for sub clinical endometritis positive cows.

The horizontal line in the box was median (The middle of the entire list of numbers). Down edge of the box was first quartile (The middle number in the first half of the data set) and up edge of the box was third quartile (The middle number in the second half of the data set). Median, first quartile, and third quartile of pregnant cows are lower than those of non-pregnant cows.

The descriptive statistics for pregnancy were shown in Table 2, separated by different amount of neutrophils. The receiver operating characteristics (ROC) analysis revealed that the best cut off point (Based on likelihood of pregnancy) was as follows: cows with $< 3\%$ neutrophil considered normal and cows with $> \text{ or } = 3\%$ neutrophil considered to have SE. Based on this cutoff point, the overall incidence of SE in dairy cows was 46% (67/147) in this study. Sensitivity and specificity are 81.3 and 97.2%, respectively, based on the selected cut off point for like likelihood of pregnancy. Subclinical endometritis had a negative impact on conception rate in the first AI after samplings, which were 10% for cows with SE and 55% for cows without SE.

In the present study endometrial cytology revealed that the PMN count of 3% and above was suggestive of subclinical endometritis. The samples which ranged from 3% to 15% of PMN cells could be correlated with subclinical cases of endometritis. Hence, the endometrial samples which contain PMN cells of 3% and above were considered as positive for subclinical endometritis.

Table 1 - Descriptive statistics for the number of neutrophil.

Amount of neutrophil	Frequency	Percentages
0	81	55.11
1	2	1.36
2	1	0.68
3	8	5.44
4	9	6.12
5	3	2.04
6	5	3.40
7	2	1.36
8	6	4.08
9	4	2.72
10	3	2.04
11	2	1.36
12	5	3.40
13	6	4.08
14	7	4.77
15	3	2.04
Total	147	100

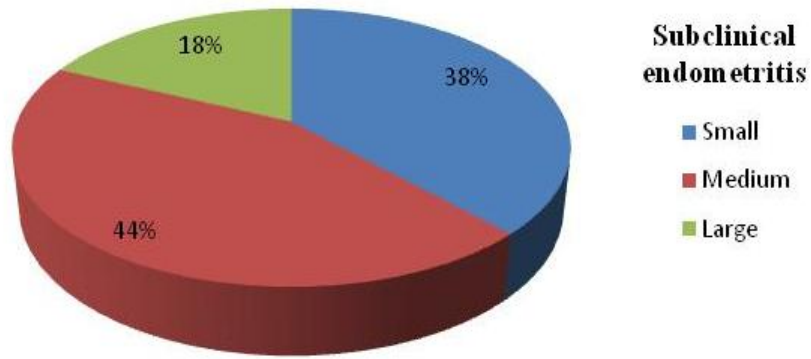


Figure 1 - Percentage of subclinical endometritis in different scale of farms.

Table 1 - The descriptive statistics for pregnancy, separated by neutrophil percentages

Amount of neutrophil	Pregnancy	Negative (-)	Positive (+)	Total
0		13	68	81
1		1	1	2
2		-	1	1
3		7	1	8
4		8	1	9
5		3	0	3
6		5	0	5
7		2	0	2
8		6	0	6
9		4	0	4
10		3	0	3
11		2	0	2
12		5	0	5
13		6	0	6
14		7	0	7
15		3	0	3
Total		75	72	147

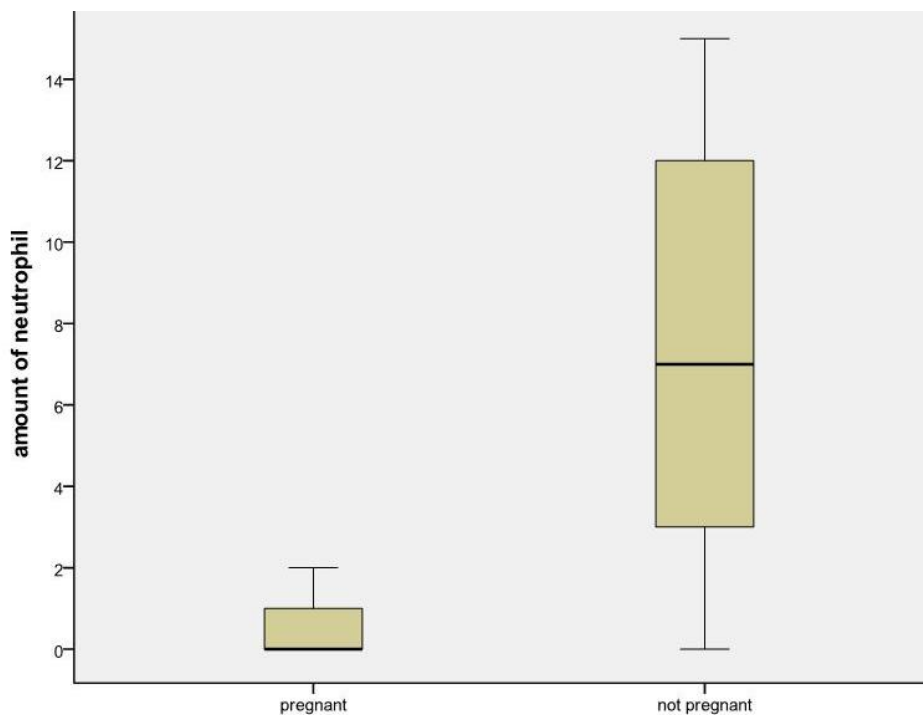


Figure 2 - The correlation between pregnancy and the amount of neutrophils.

DISCUSSION

In the present study on endometrial cytology has shown that the PMN count of 3% and above was indicative of subclinical endometritis. Similarly, in subclinical endometritis, the PMN cells were found to range from 4% to 18% (Barlund et al., 2008). Gabler et al. (2009) also reported that with ≥ 5 % PMN cells in the endometrial samples were considered as subclinical endometritis. Those earlier studies clearly indicated that 4% and 5% of PMN cells in endometrial cytological samples might be considered as "cytological marker" or "cytological indicator" for diagnosing subclinical endometritis, while in the present study PMN count of 3% and above may be considered as subclinical endometritis indicated.

The current study revealed an overall incidence of 46% (67/147) of subclinical endometritis infection in dairy cows with a PMN level of ≥ 3 neutrophils in the uterine sample which is set as a threshold value indicative for SCE which was in accordance with the earlier studies by Belachew and Fekadu (2009) in DebreZeit when prevalence of 47.5% and 30.5%. However, in the cited study samples were taken 4 and 8 weeks postpartum period separately whereas samples in this study were taken 40-60 days postpartum cows together. Gilbert (2006) in USA reported a prevalence of subclinical endometritis of 53% at 40 to 60 days postpartum and Couto et al. (2009) in Québec which was the prevalence of subclinical endometritis of 56%. In other studies, subclinical endometritis has been reported as 43% for cows between 20 and 33 days in milk, 45% for cows between 34 and 47 days in milk (Kasimanicham et al., 2005). The reason for the difference in the prevalence of SCE observed in the current study and earlier studies could be due to the difference in the management system of dairy cows.

The prevalence of subclinical endometritis is very variable and depends on the diagnosis technique; the DIM of the genital examination and the statistical method used to determine the cut-off point of the neutrophils ratio obtained from endometrial cytology (Guidry, 1976). The incidence of SCE in this study was 46% also higher than the prevalence of 13.4% SCE reported by Kaufmann et al. (2010) in Germany. However, samples in this study were taken 4 up to 8 weeks postpartum period. The higher incidence of SCE in this study compared to the above cited study could be the difference in the time of sampling. The incidence of SCE in primiparous dairy cows was 40.38% which is lower than multiparous dairy cows of 48.42%. This disagreed with Belachew and Fekadu (2009) who have reported that in DebreZeit in which first calf heifers seemed to have a tendency for SCE more often than multiparous cows at week 8 postpartum. Drilich (2006) has also reported a higher prevalence in primiparous cows which may be due to less exposure of their uterine environment to microorganism. Kaufmann et al. (2010) reported that in which the prevalence of SCE was in primiparous cows 7.8% than in multiparous cows 15.2%.

This study also shows a negative effect of subclinical endometritis on pregnancy; out of 67 subclinical endometritis positive dairy cows only 7(9.72%) cows were pregnant. This is in agreement with Belachew and Fekadu (2009) who reported 15.3% were pregnant from DebreZeit.

CONCLUSIONS AND RECOMMENDATIONS

This study revealed that subclinical endometritis was more prevalent in MSDF followed by LSDF and SSDF. The percentage of neutrophils was lower in the cows that became pregnant in the first AI. An increase in the percentage of neutrophils decreased the likelihood of pregnancy. The best cut off point (based on likelihood of pregnancy) of healthy cows was found to be $< 3\%$ neutrophil and cows with $> \text{or} = 3\%$ neutrophils had subclinical endometritis.

Subclinical endometritis subsequently decrease pregnancy in cows. So, herds should be managed properly after postpartum. The results of this study indicate that endometrial cytology can be a useful technique in identification of cows with subclinical endometritis.

DECLARATION

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Conflict of interest

The author declare that there is no conflict of interest.

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EFFECT OF HATCH WINDOW UPON INTESTINAL DEVELOPMENT, CHICK QUALITY, POST HATCH PERFORMANCE ACCORDING TO ROSS-308 BROILER BREEDER AGE

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✉ Supporting Information

ABSTRACT: This study was carried out to find out the effects of breeder age on incubation results, intestinal development during hatch window, chick quality and first week broiler performance. A total of 259280 eggs were obtained from commercial Ross-308 broiler breeder flocks at 32 and 52 weeks old, which were equally divided on the basis of breeder age in two groups, group A (young 32 weeks) and group B (old 52 weeks age). These 32 and 52 weeks old flocks, 30% and 7.0% were hatched 24h before pull time and 50% and 60% were hatched 12h before pull time, respectively. Hatchability of fertile eggs and hatchability of total eggs were found higher in 32 weeks old flock than the other. The chick hatch weight was determined as 39.5g and 42.1g in 32 and 52 weeks old flocks, respectively. Chick weight/initial egg weight rate was found to be higher as 67.3% in the 32 weeks old flocks. On hatching day, chick length was also higher in 52 weeks old flock. Relative yolk-free chick weight was higher in 32 weeks old (86.0%) flock than the other (83.0%). Intestine weight rate was higher as 5.24% in chicks from 32 weeks old than the other (4.96%). At one week of age, the body weights and weight gains were 167.5g and 162.7g, and 128g and 120.6g in 32 and 52 weeks old flocks, respectively. Higher mortality ratio as 1.9% was observed in 52 weeks old flock. In conclusion, intestinal development during hatch window, incubation parameters, chick quality and first week broiler performance is affected by breeder age.

Keywords: Incubation duration, Intestinal development, Hatch window, Post-hatch performance, Ross-308 broiler breeder

INTRODUCTION

Poultry is the second largest industry of Pakistan which plays a dynamic role in *Gross domestic product* GDP of country (Hussain et al., 2015). The value of chicken is further emphasized by its production ability and the increasing demand of its products (Anonymous, 2011).

The different phases, from rearing of grandparents stock till the broilers and eggs are marketed, have their own significance (Khan, 2011). Chick quality has great importance for broiler breeder producer who is paid based on number of saleable chicks and also broiler producer who wants high-quality, fast growing and uniform broilers (Yousaf et al., 2017). The chick quality is affected by some factors, such as breeder age, egg size, hatching time and incubation conditions (Jabbar and Yousaf, 2017). The operation and management of hatchery is vital towards the development of quality day old chicks. Various breeding practices and handling of eggs from egg laying to hatching of egg have an influence on the hatchability, particularly storage condition temperature, along with the age of breeding flock have been the most common variable used to manipulate the fertility, hatchability, livability and consequently effect on the quality of day old chick (Koka, 2002). The studies have declared that hatching egg quality and incubation conditions significantly influence the post-hatch performance of broilers (Almeida et al., 2006; Jabbar and Yousaf, 2017; Yousaf et al., 2017). The incubation period of chicken (*Gallus gallus*) embryo is approximately (506 hours) 21.08 days including drying down, and the gap among first to last chick hatch time is approximately 12 to 24 hours (Tong et al., 2013; Van de Ven et al., 2011). This time interval between first and last chick hatch is called

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“Hatch Window” (Romanini et al., 2013). In commercial hatcheries incubation times of chicken is approximately 504 hours (Almeida et al., 2006). The large scales of chicks pulling are extended up to 510 to 526 hours (Laughlin et al., 2007). Chicks that remained longer in the hatcher show worse live performance. The time period in hatcher after hatching is critical for the development of the gastrointestinal systems and nutrient absorption (Yalcin et al., 2013). Post hatching fasting impairs chick’s weight gain and breast muscles deposition capacity (Careghi et al., 2005; Halevy et al., 2000). During the first days of the chick’s life, the small intestine grows five times faster than the rest of the body, and small intestine microvilli grow significantly faster in birds supplied with water and feed immediately after hatching (Almeida et al., 2006). There is a correlation between chick qualities at hatch; broiler performance (Ipek and Sozcu, 2015).

The aim of the current study was to determine the effects of breeder age on incubation results, intestinal development during hatch window, chick quality parameters and first week broiler performance. So in this study, to evaluate the development of intestine, samples were taken during hatch window, in times of 24h and 12h before pull and at hatch time (0h).

MATERIALS AND METHODS

Ethical approval

This experiment was performed according to all ethics and animal rights (Sindh Agriculture University Tandojam, Pakistan).

Experimental site

The study was carried out at Salman Poultry (Pvt) limited, Chakri hatchery Rawalpindi which is situated five km from Chakri interchange on motorway. The hatchery contains latest heating ventilation and air conditioning automation. This is the one of largest eggs capacity hatchery of south Asia, which is producing best quality of chicks through single stage incubation system (Avida G4, Chick Master USA).

Selection and handling of eggs

Eggs (52-60g) from commercial broiler breeders group A (Ross-308, 32 weeks of age) and Group B eggs (60-64g, 52 weeks of age) with broiler breeder diet with 2750 kcal ME/kg and 14.50% Crude Protein (CP) was selected for current study. Both flocks were kept under the same management conditions according to the breeding company’s recommendations. Each experimental group was consisting of 134640 eggs, which were graded upon their quality, poor shell, elongated eggs, crack were isolated, only standard eggs were set in the incubator machine Advida4 chick master USA (Yousaf, 2016). These eggs were collected at farm at 20 °C and 75% relative humidity until used in hatching trial (Yousaf et al., 2017). Before, trial eggs were fumigated with 20g KMnO₄ and 40ml formalin (40%) and 40ml of water for 100ft³ areas for 15 minutes through automatic fumigation process provided by Chick Master (Yousaf et al., 2017).

Incubation regime

Both groups were pre-heated at 82°F for five hours inside incubators. After completion of pre-warming the setter started automatically the age wise incubation stage profile (recommended by Chicks Master USA). Incubation duration for both groups was remaining same 456h in setter and 50h in hatchers (Yousaf et al., 2017).

Hatchery analyses

Before transfer to hatchers water loss of both groups was measured. Water loss was measure after 456 hours as given formula:

$$\text{Water Loss \%} = \frac{\text{Full tray weight at setting} - \text{Full tray weight at transfer}}{\text{Full tray weight at setting} - \text{Empty tray weight}} \times 100$$

Eggs from were transferred to hatchers after 456h of incubation in setter. For both groups during transfer from setter to hatchers candling was performed through candling tables. As hatch out immediately the chick’s weight and yield was measure through electrical weight balance by using following formula:

$$\text{Chick Yield \%} = \frac{\text{Weight of chicks} \times 100}{\text{Egg weight}}$$

Unhatched eggs were opened to macroscopically determine fertility and embryonic mortality (early, middle- and late-term embryonic mortality) (Table 1).

Hatch window and intestine analysis

The hatched chicks in time of 482 h, 492h and 503-506 h were counted, and the rate of hatching chicks according to these times was calculated. In these times, after chicks were counted, chicks were transferred to another hatching basket. During hatch window, to determine development of intestine, chicks (n=20 chicks/sampling time/breeder. age) were randomly sampled in the sampling times [24h before pull time (-24 h), 12h before pull (-12 h) and at hatch (0h)]. In sampling times for -24h and also -12h before pull time, chicks were randomly sampled from hatcher. The sampled chicks were weighed and euthanized by cervical dislocation, and then the intestine was dissected. The intestine weight and length were measured, and the ratio of intestine weight to chick weight was calculated. The length of intestine was measured from the beginning of small intestine to the end of the cloaca.

Hatch pulling

Hatch pulling was performed through conventional method of hatch pulling in Pakistan. First pull at 494h (456h in setters and 38h in hatchers). For second hatch pull the remaining pips and unhatched eggs were again shifted to hatcher for next 12h. After 12h again pulling of un-hatch eggs was performed to determine the effects of incubation results on intestinal development during hatch window. Grading of chicks was performed on conveyer, automatic grading table. Only stranded (shining eyes, soft legs and nose, healed naval and healthy chicks) were shifted to chick's box after counting, while under weight, weak, and unhealed naval chicks were removed as international standard.

Analysis of residual yolk in chicks

Chicks from each group were measured for chick weight and chick length and then euthanized by cervical dislocation to determine residual yolk sac weight and yolk-free chick weight. Chick length was measured from the tip of the beak to the tip of the longest toe by placing the chick face down on a flat surface and straightening the left leg.

Delivery to poultry house

Total n=56000 (group A; n=28000, group B; n=28000) day old chicks were send to Salman broiler farm Khilari-Chakri, Rawalpindi. Environmentally control vehicles (75 °F temperatures, 65% humidity) are used to deliver the chicks to control poultry house in the 102 chicks/box with dimensions of plastic chick's box (27×19.5×6.5 inches). Poultry house condition was remained same for both groups. At farm, chicks of both groups were offered water and feed diets (3020 Kcal ME/kg, 22% CP) ad libitum. The diet was formulated according to the recommendations of the NRC (1994) using windows user-friendly feed formulation (WUFFDA) software program. Intake of feed and water was record daily, while body weight and total feed consumed were recorded on weekly basis. After seven days chicks 'weights were measured for both group, mortality was recorded during the first week. For ventilation viper touch (Big Dutchman, Co., Germany) system was installed.

Statistical analyses

All data were analyzed by using Statistical Analysis System package software (SAS version 9.2, SAS Institute Inc., Cary, NC, USA). All means were compared using Duncan's multiple range test and results were presented as mean± SEM (standard error of mean). Results were considered significant if exist (P<0.05).

RESULTS AND DISCUSSION

The hatch window results of 32 and 52 weeks old broiler breeder flocks are presented in table 1. Hatch window was found that 30% and 7% of the chicks were hatched in the time of 482 h,

50% and 60% of the chicks between 492h, and 23% and 27% of the chicks between 504h to 506h in 32 and 52 weeks old broiler breeder flocks, respectively. Incubation period was completed in 504h in 32 weeks old breeder flock, whereas it was completed in 510h in 52 weeks old breeder flock. The effects of breeder age on incubation results and cloacal temperature are presented in table 2. The effects of breeder age on egg weight was found to be significant (P=0.001). Egg weight was 57.1 g and 63.7 g in 32 and 52 weeks old flocks, respectively. Fertility was similar in breeder age groups, whereas hatchability of fertile eggs and hatchability of total eggs were found to be higher in 32 weeks old flock than the other (P=0.001). Hatchability of fertile eggs and hatchability of total eggs were 90.1% and 87.67% in 32 and 52 weeks old flock, respectively. Early, middle and late-term embryo dead and cull chick rate were found to be higher in 52 weeks old flock (P=0.001). The cull chick rate was 0.81% and 1.7% in 32 and 52 weeks old flocks, respectively. Water loss was found higher with a rate of 13.1% in 52 weeks old flock than the other (P=0.001). Chick hatch weight was 39.5g and 42.1g in 32 and 52 weeks old flocks, respectively (P=0.015). Chick weight/initial egg weight rate was found to be higher with a value of 68.3% in the 32 weeks old flock (P=0.035). The cloacal temperature was found to be similar between flocks (P=0.058).

The effects of breeder age on the development of intestine development during hatch window are presented in table 3. The intestine weight rate was only found to be significant in breeder age groups. It was higher with a value of 5.24% in chicks from 32 weeks old flock than the other (4.96%; P=0.046).

During the hatch window, the chick weight, the weight and length of intestine and the intestine weight ratio were similar in sampling times. The effects of breeder age on the chick weight, chick length, residual yolk sac weight, relative residual yolk sac weight, yolk-free chick weight and relative yolk-free chick weight are presented in table 4. On hatching day, chick weight and length were found as 39.5g and 42.1g, and 18.5cm and 19.4cm in 32 and 52 weeks old flocks, respectively. Higher residual yolk sac weight and relative residual yolk sac weight were observed in 52 weeks old flock with a value of 7.1g and 18.2%. Although yolk-free chick weight was similar between flocks, relative yolk-free chick weight was higher in 32 weeks old flock (86.0%) than the other (83.0%).

The effects of breeder age on the post-hatch first week broiler performance parameters are presented in table 5. The initial body weight on day one was higher in the 52 weeks old flock (42.1g) than the 32 weeks old flock (39.5g). At one week of age, the body weights and body weight gains were determined as 167.5g and 162.7g, and 128g and 120.6g in 32 and 52 weeks old flocks, respectively. Feed consumption and feed conversion rate were similar between treatments for the first week. Higher mortality ratio as 1.9% was observed in 52 weeks old flock.

Table 1 - Hatch window results of flocks at different ages

Groups	Chicks hatch out after incubation duration (%)		
	After 482h	After 492h	After 506h
A (32 weeks)	30%	50%	23%
B (52 weeks)	7%	60%	27%

Table 2 - The effects of breeder age on incubation results

Incubation results	32 week	52 week	P Value
Egg weight (g)	57.1 ± 1.4	63.7 ± 1.5	0.001
Fertility (%)	95.2 ± 0.8	96.0 ± 0.9	0.001
Hatchability of fertile eggs (%)	90.1 ± 2.5	87.67 ± 2.1	0.001
Hatchability of total eggs (%)	88.3 ± 1.3	84.2 ± 2.2	0.001
Early term embryo dead (%)	2.2 ± 0.5	4.0 ± 1.4	0.001
Middle-term embryo dead (%)	0.88 ± 0.4	1.7 ± 0.4	0.001
Late-term embryo dead (%)	4.2 ± 1.4	6.6 ± 1.5	0.001
Cull chicks (%)	0.81 ± 0.3	1.7 ± 0.2	0.001
Water loss (%)	11.2 ± 1.1	13.1 ± 1.2	0.001
Chick hatch weight (g)	39.5 ± 1.2	42.1 ± 1.0	0.015
Chick weight/initial egg weight (%)	67.3 ± 1.2	65.2 ± 1.4	0.035
Total incubation period (h)	504	510	–
Cloacal temperature (°C)	38.8 ± 1.7	39.0 ± 1.5	0.058

Table 3 - The effects of breeder age on intestinal development during hatch window

Breeder age (weeks)	Chick weight (g)	Intestine weight (g)	Intestine weight rate (%)	Intestine length (cm)
32	39.5	2.07	5.24	39.9
52	42.1	2.09	4.96	41.2
Sampling time (h) before hatch				
-24	40.8	2.14	5.24	41.3
-12	40.6	2.07	5.09	41.1
0	40.1	2.06	5.13	41.00
Age (weeks)×Time (h)				
32 × -24	39.7	2.11	5.31	40.7
32 × -12	39.4	2.08	5.27	40.5
32 × 0	39.2	2.07	5.28	41.00
52 × -24	42.3	2.13	5.03	41.6
52 × -12	39.9	2.11	5.28	41.4
52 × 0	40.00	2.12	5.3	41.5

Table 4 – Effect of breeder age upon yolk in Ross-308 chick

Characteristics	32 week	52 week
Chick weight (g)	39.5±0.56	42.1±0.52
Chick length (cm)	18.5±0.3	19.4±0.3
Residual yolk sac weight (g)	5.7±1.15	7.1±1.75
Relative residual yolk sac weight (%)	15.3±2.5	18.2±2.1
Yolk-free chick weight (g)	32.5±1.5	33.3±1.7
Relative yolk-free chick weight (%)	86±0.5	83±0.5

Table 5 - Post hatch performance parameters of first week Ross-308 broiler breeder ages

Parameters	32 week	52 week
Initial weight, g/bird	39.5± 0.2	42.1± 0.2
Body weight, g/bird	167.5± 1.2	162.7± 1.2
Body weight gain, g/bird	128± 2.2	120.6± 2.5
Feed consumption, g/bird	116 ± 3.2	118± 3.5
Feed conversion ratio	0.95 ± 0.5	1.05 ± 0.5
Mortality %	0.85	1.9

This study has investigated the effects of breeder age on incubation results, intestinal development during hatch window, chick quality parameters and first week broiler performance. In 52 weeks old flock, the actual hatching rate at 12h and 24h of pull time was lower than expected, so it caused longer time range between the first and last hatched chicks. In other studies, hatch window range was found as 24–48h (Decuyper et al., 2001), 28h (Careghi et al., 2005) and 30h (Van De Ven et al., 2011). Similarly, it was observed that eggs laid by 32weekoldbreeders hatched 9h early than those laid by 52weekoldbreeders (Pedroso et al., 2005). In this study, egg weight was 57.1g and 63.7g in 32and 52 weeks flocks, respectively. The time required for hatching increased in heavy eggs as compared to lighter eggs of older age breeders.

In this study, the hatchability of fertile eggs and hatchability of total eggs of old breeders' eggs were lower than those of young breeders, which were found significantly different. In this study, higher ratio of embryo mortalities was observed in older flock. Rate of cull chicks (%) was found to be higher as 1.7% in 52 weeks old group. The reason of higher rate of cull chicks in 52 weeks old group could be attributed to longer hatch window. The average egg weight loss should be between 11.5% and 13% to obtain the highest hatchability of chicken eggs. Weight loss was higher as 13.1% in 52 weeks old flock. Chick hatch weight and chick weight/initial egg weight rate were found to be significant. Chick hatching weight was found higher in 52 weeks old flock, and chick weight/initial egg weight rate was affected by breeder age. Embryo uses the nutrients from the yolk sac to initiate body growth (Meijerhof, 2009), for development of the small intestine and other organs. Residual yolk sac comprises approximately 14% of the chick's body weight at the time of hatching (Meijerhof, 2009). Before hatching, absorption of the yolk sac into the abdomen of the embryo provides nutrients for the chicks during the first few days of life. Chick weight is a combination of the real chick weight and the remaining yolk residual. On the hatching day, the residual yolk sac weight and relative residual yolk sac weight were found higher as 7.1g and 18.2% in old flock, respectively. After subtracting yolk sac weight from chick weight, there were no differences between groups. Relative yolk-free chick weight was found to be higher in young flock. The quality of the day old chick is important for a good start of the chick and also for the final performance of broiler (Meijerhof, 2009). Breeder age, egg weight, egg age, climatic conditions of both hatchery spaces and incubators, and some other factors predominantly affect hatch window and therefore chick quality (Vargas et al., 2009). On hatching day, chick weight and length were found as 39.5g and 42.1g, and 18.5cm and 19.4cm in 32 and 52 weeks old groups, respectively. In this study, heavier and longer chicks in old flock were resulted from heavier egg weight. It was stated that chick length has a substantially higher positive correlation with broiler performance than day old chick weight, especially when corrected for egg size (Meijerhof, 2009). Initial body weight was lower in young flock, after a week post-hatch, body weight and body weight gain were found higher than old flock. It could be explained by yolk sac absorption, and development of intestine of the chicks in this group was higher than the other. Intestine weight was heavier in chicks from young than from old flock in this study. Feed consumption and feed conversion rate were not influenced by breeder age. The mortality during first week was found significantly higher as 1.9% in 52weeks old flock. The time range after hatching is vital for the development of immune and gastrointestinal systems. While early term chick feeding stimulated the development of bursa and production of lymphocytes (Bigot et al., 2001), long fasting times stimulate corticosterone production that has a

strong inhibitor effect on immune cells. These cause a decrease in growth rate and an increase in early term chick mortalities (Decuypere et al., 2001).

CONCLUSION

Intestinal development during hatch window, incubation parameters, chick quality and first week broiler performance is affected by breeder age. It is recommended that the older flock eggs chicks had better development of intestine as compared to younger flock age eggs. The health status of younger broiler breeder eggs broiler perform very best in overall conditions.

DECLARATIONS

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Competing interests

The authors have declared that no competing interest exists.

Author's contribution

Dr. A.Yousaf was the main researcher, Dr. N.Rajput was research coordinator, Dr. A.Memon was research supervisor, Dr. R.Shahnawaz revised the article, Dr. G.NazJagirani and Dr. F.Habib contributions in statistics while Dr. S.Rajpar and Dr. M.Sarwar assisted in results analysis and other activities related to the research.

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EFFECT OF AGE ON CERTAIN URINE PARAMETERS OF YOUNG CAMELS (*Camelus dromedarius*)

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✉ Supporting Information

ABSTRACT: The objective of this study was to identify reference values for certain urine parameters in relation to the age in young dromedary camels. Twenty one healthy young female camels (age <3-5 months) of Arabi local breed were divided into three groups (≤ 3 m: n=6, 3-4 m: n=8 and 4-5 m: n=7). Jugular venous blood samples were collected and used to determine electrolytes and creatinine concentration, whereas the urine samples were used for the determination of urine pH, urine osmolality, and for the calculation of fractional excretion of electrolytes. The mean value of urine pH was 7.9 ± 0.5 (reference range: 7.2-8.8 and the percentile range: 7.3-8.2) and the urine osmolality in young camels was 978.9 ± 468 (reference range 235-1819 mOsmol/kg) for all age groups. Fractional excretion of Na^+ (FE_{Na^+} %) showed lower mean values compared to the fractional excretion of K^+ and Cl^- . The age had a significant ($P < 0.05$) effect only on urine osmolality in camels of 4-5 months. The identified reference values of certain urine parameters can be used for the clinical diagnosis of renal diseases in growing animals. The data could be utilised for the clinical monitoring of the physiological and pathological status of animals fed strong electrolytes such as Na^+ , K^+ and Cl^- .

Keywords: Age, Dromedary camels, Reference values, Urine parameters

INTRODUCTION

The kidneys are well known as being the main organs in the body responsible for the regulation of the extracellular electrolyte concentration and acid-base status of mammals by adjusting urine electrolyte excretion to maintain constant systemic pH (Bevenssee and Boron, 2008). Evaluation of the renal function has been accomplished with the analysis of blood and urine to compare the concentration of electrolytes and creatinine in blood and urine, which is known as Fractional excretion of certain electrolyte ($\text{FE}_{\text{electrolyte}}$).

The FE of electrolytes is a simple, inexpensive method that has been shown to be a reasonable indicator for renal clearance of electrolytes in animals (Lefebvre et al. 2008; Braun and Lefebvre, 2008). Therefore, measurement of $\text{FE}_{\text{electrolyte}}$ can be used as a potential tool to assess the kidney function (Lefebvre et al., 2008) and as a diagnostic and prognostic application to renal dysfunction (Troia et al., 2018). In veterinary practice, the FE of electrolytes has been calculated by comparing the amount of the substance excreted in the final urine with the amount filtrated through the glomerulus (Fleming et al., 1992).

Under physiological conditions, a considerable variation on the $\text{FE}_{\text{electrolyte}}$ in relation to the age has been reported previously in calves (Henniger et al., 2013), dogs (Laroute et al., 2005), cats (Hoskins et al., 1991) and horses (Edwards et al., 1990). Many researchers have stated that the $\text{FE}_{\text{electrolyte}}$ has been used to investigate and validate the evaluation of dietary adequacy, the response to treatment and the impaired renal function in animals (Fleming et al., 1991; Fleming et al., 1992; Ulutas et al., 2003; Ulutas and Sahal, 2005). In clinical practice, $\text{FE}_{\text{electrolyte}}$ has been applied to cattle before calving in order to reduce their susceptibility to metabolic diseases mainly milk fever (Ulutas et al., 2003; Mellau et al., 2002) and in dairy cows with left displaced abomasum (Perotta et al., 2018). In camels, $\text{FE}_{\text{electrolyte}}$ has been applied to camels with experimentally induced metabolic acidosis (Elkhaier, 2008).

Measurement of urine pH has been used as a practical and inexpensive method for monitoring the effectiveness of dietary cation-anion difference (DCAD) in lactating cows (Hu and Murphy, 2004) and to assess renal function after acid-load with NH_4Cl (Elkhaier and Hartmann, 2010). Lubetskaya and Melnichuk (1999) used urine pH as an index for calculating the amount of bicarbonate for the treatment of acidotic calves. On the other hand, Mellau et al. (2002)

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used urine pH as an indicator to assess the influence of short-term anion salt exposure in cows with experimentally induced hypocalcaemia. A close relationship has been reported previously between urine pH and diet content in cattle (Hu and Murphy, 2004; Hu et al., 2007). However, to our knowledge no published data has been available regarding the monitoring of urine parameters in young camels of different ages. Consequently, the study was aimed to identify reference values for certain urine parameters in young dromedary camels in relation to their age.

MATERIALS AND METHODS

Ethical approval

The study was approved by The Sudan Veterinary Council (EA/0030/2018).

Animals and Management

Twenty one healthy young female camels of Arabi local breed (Aged <3-5 months) were divided into three groups (≤ 3 m: n=6, 3-4 m: n=8 and 4-5 m: n=7). The young female camels were housed in an outdoor environment in a shaded corral in Camel Research Centre farm (Faculty of Veterinary Medicine, University of Khartoum, Sudan). The animals were maintained on grazing and browsing trees and shrubs in the vicinity of the Camel Centre and occasionally received fresh grass (*Sorghum lactabicolor*, Abu 70) and concentrate supplements, which was offered daily with free access to fresh water.

Sample collection and laboratory analysis

The experiment was conducted during the period January 2007 to January 2008. Jugular venous blood samples were collected in the morning before starting feeding by jugular venipuncture using plastic syringes (7.5 mL, Pirmvetta®, Laboratory Technique, GmbH, Germany). The blood samples were centrifuged and the serum was collected in sterile containers for further analysis of serum electrolytes and creatinine concentration using a biochemical analyser (Roche Hitachi Modular, Roche) (Table 1).

A volume of 100 ml urine samples were collected in sterile containers via free catch or by perineal or preputial stimulation of the camels at the same time of the blood collection. Ten ml of fresh urine samples were used to determine urine pH using a pH meter (InoLab, Scientific Technical Workshops, Weilheim, Germany) (Table 1). The pH-meter was calibrated using a two-point calibration with pH 10.0-5.0. Urine osmolality was determined by the Osmometer (OSMOMAT 030, gonotec, Germany) (Table 1). The rest urine samples were used for the determination of electrolytes and creatinine concentration using a biochemical analyser (Roche Hitachi Modular, Roche) (Table 1).

Table 1 - Blood and urine parameters and methodology of measurements

Parameter	Methodology	Equipment	CV (%)
Urine pH	Ion selective electrode	pH meter	
Osmolality	Freezing point-depression measurement	OSMOMAT 030	1.3
Na ⁺	Ion-selective electrode measurement	Roche Hitachi Modular, Fa. Roche Diagnostics	1.26
K ⁺			1.77
Cl ⁻			2.39
Creatinine	Kinetic colour test after Jaffé		2.59

CV: Coefficient of variation

Electrolytes and creatinine concentrations were used for the calculation of fractional excretion of electrolytes as described by Fleming et al. (1991):

$$FE_{\text{electrolyte}} (\%) = \frac{\text{Urine-[electrolyte]}}{\text{Plasma-[electrolyte]}} \times \frac{\text{Plasma-[creatinine]}}{\text{Urine-[creatinine]}} \times 100$$

Statistical analysis

Statistical analysis was performed using SPSS for Windows version 20.0. The normal distribution of the individual data was determined using a One-Sample Kolmogorov-Smirnov adjustment test. The statistical analysis of certain urine parameters were estimated using descriptive statistics procedures of the same programme. ANOVA tests (Levine's Test and Post Hoc Test) were used to assess the possible significant differences between the age groups. The mean difference was considered significant at $P \leq 0.05$.

RESULTS

The statistical data of certain urine parameters shown in table 2 demonstrated that the mean values of urine pH and urine osmolality of young camels were 7.9 ± 0.5 (Reference range: 7.2– 8.8) and 979 ± 468 mOsmol/kg (Reference range: 235-1819 mOsmol/kg), respectively. The values mean values of FE_{electrolytes} (%) of young camels for Na⁺, K⁺ and Cl⁻ were 0.07 ± 0.04 , 13.3 ± 5.5 and 1.4 ± 0.8 , respectively.

The detailed results shown in table 3 indicated that the age had no significant effect on the most of urine parameters investigated. Urine osmolality showed a gradual decrease with advancing age; however, urine osmolality of the young camels (4-5 months) showed higher ($P < 0.01$) values of 1179 ± 244 mOsmol/kg compared to the other age groups (891 ± 362 and 870 ± 496 mOsmol/kg).

Items	Statistical values	Young camels
Number of animals		21
Age		3 - 5 m
Urine pH	$\bar{x} \pm S$	7.9 ± 0.5
	Reference range ¹	7.2-8.8
	Median	7.96
	(1.-3. Quartile)	7.3-8.2
Osmolality (mOsmol/kg)	$\bar{x} \pm S$	979 ± 468
	Reference range ¹	235-1819
	Median	1112
	(1.-3. Quartile)	559-1292
FE _{Na+} (%)	$\bar{x} \pm S$	0.07 ± 0.04
	Reference range ¹	0.03-0.2
	Median	0.06
	(1.-3. Quartile)	0.05-0.1
FE _{K+} (%)	$\bar{x} \pm S$	13.3 ± 5.5
	Reference range ¹	1.9-3.9
	Median	12
	(1.-3. Quartile)	12-16
FE _{Cl-} (%)	$\bar{x} \pm S$	1.4 ± 0.8
	Reference range ¹	0.2-3.2
	Median	1.3
	(1.-3. Quartile)	0.8-1.9

$\bar{x} \pm S \times 1.96$ indicated the lower and the upper limits; d: day; m: month(s); $\bar{x} \pm S$: mean \pm SD (standard deviation)

Items	Experimental young camels		
	6	8	7
Number of animals	6	8	7
Age	≤ 3 m	3-4 m	4-5 m
Urine pH	$7.9^a \pm 0.5$	$8.1^a \pm 0.6$	$7.7^a \pm 0.8$
Urine osmolality (mOsmol/kg)	$891^a \pm 362$	$870^a \pm 496$	$1179^b \pm 514$
FE _{Na+} (%)	$0.06^a \pm 0.03$	$0.07^a \pm 0.02$	$0.08^a \pm 0.06$
FE _{K+} (%)	$10.8^a \pm 4.2$	$14.2^a \pm 5.2$	$14.3^a \pm 6.9$
FE _{Cl-} (%)	$1.2^a \pm 0.8$	$1.2^a \pm 0.7$	$1.8^a \pm 0.8$

m: month; Means within the same row bearing different superscripts are significantly different at $P \leq 0.05$

DISCUSSION

To our knowledge no published data is available regarding the reference values of certain urine parameters in camels less than 6 months of age. Consequently, the present study was proposed to identify a reference range for certain urine parameters in young camels for subsequent use. The statistical data obtained in the present study for the urine pH of 7.9 ± 0.5 demonstrated the significant impact of the nutritional status of the animal on urine pH because the young camels fed milk and hay. Therefore, the result can be considered as a physiological or renal response to the diet shift from milk to hay.

The results presented in table 2 showed that the values of FE_{electrolytes} of young camels higher than those that of young ruminants mainly calves (Elkhair, 2008). This pattern of response could be explained by the specific morphometric features of the camel's kidney such as a long loop of Henle, a well-developed medulla and lower glomerular filtration rate (El Bahri et al., 1999; Abdalla and Abdalla, 1979). Moreover, the camel's kidney is known to have a strong capacity of water reabsorption and high ability to eliminate very concentrated urine (Abdalla and Abdalla, 1979; Ouajd and Kamel, 2009). Therefore, the higher mean values of FE_{Na+} and FE_{K+} of young camels obtained in the present study could be explained by the effects of renin-angiotensin-aldosterone system on the renal secretion of these electrolytes (Brobst, 1986), which influenced urine osmolality. Furthermore, many investigators stated that urinary excretion of electrolytes is a complex process involving filtration, reabsorption and secretion in various areas of the nephron (Koeppen and Stanton, 2013). Each of these processes may be influenced by numerous factors such as age (Hartmann et al., 1987), dietary intake (Hu and Murphy, 2004), the status of body fluids and the activity of various hormones (Koeppen and Stanton, 2013). Therefore, critical attention of this situation should be considered in fluid therapy of these animals.

The detailed results shown in table 3 indicated that the age had no significant effect on the most of urine parameters investigated. Urine pH of the calves showed approximately similar mean values ranging from 7.7-8.1 for all age groups and appeared to be neutral or alkaline urine. In contrast, variations on urine parameters have been reported previously in relation to the age in other animal species (Henniger et al., 2013; Elkhair, 2008; Laroute et al., 2005; Hoskins et al., 1991; Edwards et al., 1990).

The results obtained in the present study indicated that urine osmolality decreased with advancing age. This pattern of response can be attributed to the fact that the renal function can be modified with advancing age to satisfy the demands of animal's growth and/or depend on their nutritional status.

CONCLUSION

Urine pH, urine osmolality and urinary FE_{electrolyte} can be used for monitoring of renal function in camels. The nutritional status of the animals has an influence on the renal function of the young camels rather than their ages. Further investigations are needed to identify a reference range for quantitative renal parameters to each age group. The results can be utilised for monitoring the effectiveness of applied fluid therapy in camels.

DECLARATIONS

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Author's contribution

The author performed the data collection and analysis. The author reviewed information, wrote up and approved the final manuscript.

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Conflict of interest

The author declares that there was no conflict of interest

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THE INFLUENCE OF FEED FORM ON BROILER PRODUCTION AND GASTROINTESTINAL TRACT DEVELOPMENT

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✉ Supporting Information

ABSTRACT: A completely randomized design experiment was conducted to assess the influence of feed form on broiler production and gastrointestinal tract development. A total of (n=315) 16 days-old Ross 308 chicks were randomly assigned to three dietary treatments (mash, crumbles and pellets) with three replicates. Weekly data indicated that birds on pellet and crumble diets had a significant ($P<0.05$) improvement on a number of production parameters than birds fed diet in a mash form. Data for average values for the whole experimental period demonstrated that the dietary treatments had significant effect on feed intake (0.001), body weight ($P=0.013$), performance index ($P=0.040$), abdominal fat weight ($P=0.010$) and carcass weight. ($P=0.001$). However, feed conversion ratio and carcass yield did not show significant ($P>0.05$) response to dietary treatments. Contrary to weekly data the averages for the whole experimental period data was dominated by birds on crumble diet with the highest feed intake, body weight, abdominal fat weight and carcass weight. Gastrointestinal tract development results indicated that dietary treatment had a significant effect on intestinal length ($P=0.015$) whereby birds that consumed diet in a mash form had superior intestinal length. The intestinal and gizzard weight parameters were not significantly ($P>0.05$) influenced by the dietary treatment. The results of the current study proved that the crumble form of a diet is the most suitable for optimum broiler production during growing and finishing stages while diet in a mash form is suitable for optimum development of gastrointestinal tract.

Keywords: Carcass yield, Crumble, Feed form, GIT development, Mash, Pellets

INTRODUCTION

Nowadays, various commercial feed mills are producing different feed forms of broiler feed for different age group of birds. Feeding of each form has its own advantages and disadvantages (Ghazi et al., 2012). The effectiveness, digestibility and conversion efficiency of different forms of feeds are also different. However, the dilemma still exists amongst many farmers in Lesotho regarding which form of feed is suitable for production at different stages of broiler growth. The majority of feeds used in commercial production of broilers is given in the form of pellets, mash or crumbles (Waldroup, 1997; Yasar, 2003; Sarvestani, 2006). Mash is form of a complete feed that is finely ground and mixed so that birds cannot easily separate out ingredients and each mouthful provides a well-balanced diet. Mash diets gives greater unification of growth, less death loss and are more economical because pellets and crumbles costs slightly more than the same ration in mash form. However, ground feed is not so palatable and does not retain their nutritive value as well as ungrounded feeds. In general, processing complete poultry feed into pellets involve preconditioning the total mixed diet which is followed by extrusion of the mash through a pellet mill die. In the recent decade, usage of poultry feed in the form of pellet have a lot of benefits such as; decreased ingredient segregation, less time and energy expended for prehension, destruction of pathogenic organism, thermal modification of starch and protein, improved palatability and high feed intake (Jahan et al., 2006). Crumble diet also is a type of feed prepared at the mill by pelleting of the mixed ingredients and then crushing the pellet to a consistency coarser than mash. Recently this form feed is becoming popular in broiler production due to its convenience of feeding.

Crumbles are particularly suitable for young broilers (Cerata et al., 2009). The feeding of meal on the other hand at this stage can limit growth. It is therefore essential to utilize the intake and growth potential of this period to the full and crumbles are particularly suitable for this. The structure of feed for broiler chickens has a strong influence on the physiological functions and development of the digestive tract. A well-developed gastrointestinal tract will lead to

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improved feed utilization and gut motility (Ferket, 2000). Therefore the main objective of this study is to determine the effect of three dietary forms on broiler production and digestive tract development.

MATERIALS AND METHODS

The completely randomized study was conducted at the National University of Lesotho farm in 2014. The University is located 34 km southeast of the capital city Maseru. The climate of this area alternates between the hot and cold months. The winter being the coldest season and is experienced from May to August and temperature may drop as low as -1°C, summer is the hottest season is experience from September to April and temperature can be as high as 28°C.

The study was made up of three dietary treatments replicated three times. The three treatments were represented by three different feed forms of broiler feeds being mash, crumbles and pellets. All the dietary treatments had similar nutritive value in terms of crude protein and metabolisable energy for all feeding phases, with the exception of feed form. The chemical composition of the feeds is illustrated in table 1. A total of 315, 16 day-old Ross 308 chicks with an average weight of 580 grams were used in the trial. Production variables including feed intake and body weight were measured on weekly basis, while feed conversion ratio and performance index were calculated from this data using the following formulas.

$$\text{Feed conversion ratio (FCR)} = \frac{\text{Feed intake (g)}}{\text{Live weight (g)}} \quad ; \quad \text{Performance index (PI)} = \frac{\text{Live weight (kg)}}{\text{Feed conversion ratio}} \times 10$$

At the end of experimental period all birds in each replicate were killed following a 6-hour fasting in order to determine carcass yield. Carcass yield was determined as the weight of the eviscerated carcass in relation to live weight after fasting. Gastrointestinal tract parameters data was collect at six weeks of age on the following parameters; gizzard weight, intestinal weight and intestinal length. Gizzard and intestinal weights were determined by weighing them on the digital weighing scale after the removal of intestinal contents. Intestinal length was measured using a measuring type. Birds were withdrawn food for 24 hours but given water in order to empty the digestive tract and to avoid meat contamination during slaughtering.

Statistical analysis

The response variables were analyzed as one-way ANOVA with three dietary treatments and weeks as the main effects using the GENSTAT C statistical package (IBM SPSS, 2011). Once differences were detected by ANOVA, means were separated using Tukey studentized range.

Table 1 - Chemical composition of dietary treatments

Nutrient	Feeding phases		
	Starter	Grower	Finisher
Crude protein (%)	22	20	18
Metabolisable energy (Kcal)	3050	3150	3200
Lysine (%)	1.20	1.10	0.90
Methionine (%)	0.48	0.44	0.38
Calcium (%)	0.96	0.91	0.90
Phosphorus (%)	0.41	0.40	0.38

RESULTS

Production and carcass parameters

The effects of feed form on broiler production results are illustrated in Figures 1 to 3 for weekly data and in Table 2 for the averages of entire experimental period. According to the results presented in Figures 1 to 3, the dietary treatments had a significant ($P \leq 0.05$) influence on feed intake, feed conversion ratio, performance index across all the weeks. Birds on treatment three (crumble diet) resulted in the highest ($P \leq 0.05$) feed intake, live weight and performance index across all weeks. Live weight results (Figure 2) illustrated a tight competition between treatment two and three whereby both treatments resulted in the similar body weights during week two and three while during the fourth week treatment two (crumble) had a marginal non-significant ($P \geq 0.05$) higher body than treatment three (pellets).

The results of the average performance during the entire experimental period (Table 2) indicated that the dietary treatments had a significant ($P \leq 0.05$) influence on feed intake, live weight, performance index, abdominal fat weight and carcass weight but did not have significant ($P \geq 0.05$) effect on feed conversion ratio and carcass yield. According

to these results birds fed crumble diet resulted in highest feed intake, live weight and carcass weight followed by birds on pelleted diet and the lowest being birds fed mash diet. Carcass parameters also followed similar trends whereby birds fed crumble diet had the highest carcass yield, carcass weight and abdominal fat while birds fed mash diet had lowest yields.

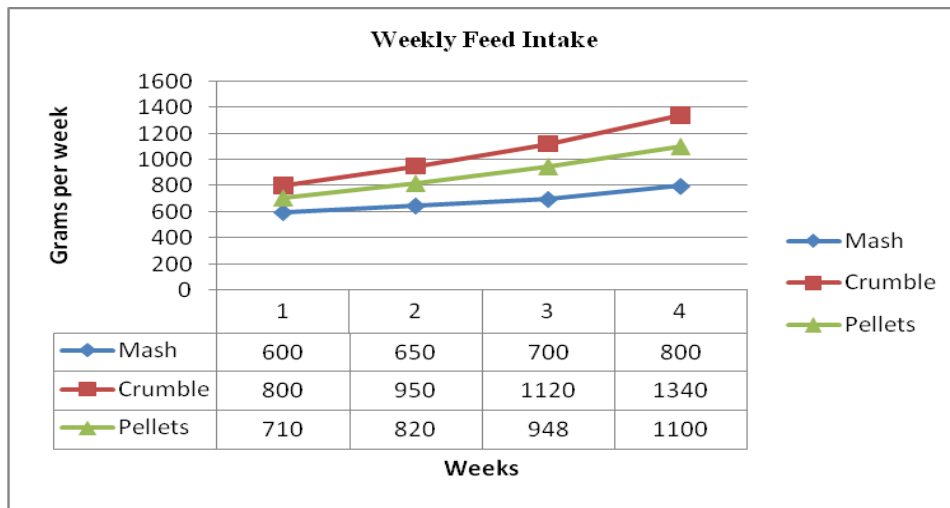


Figure 1 - Weekly feed intake

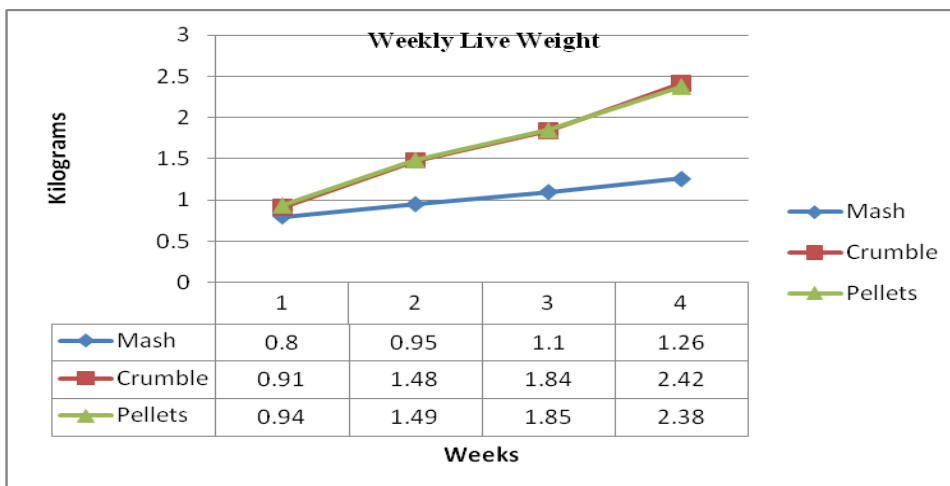


Figure 2 - Weekly live weight

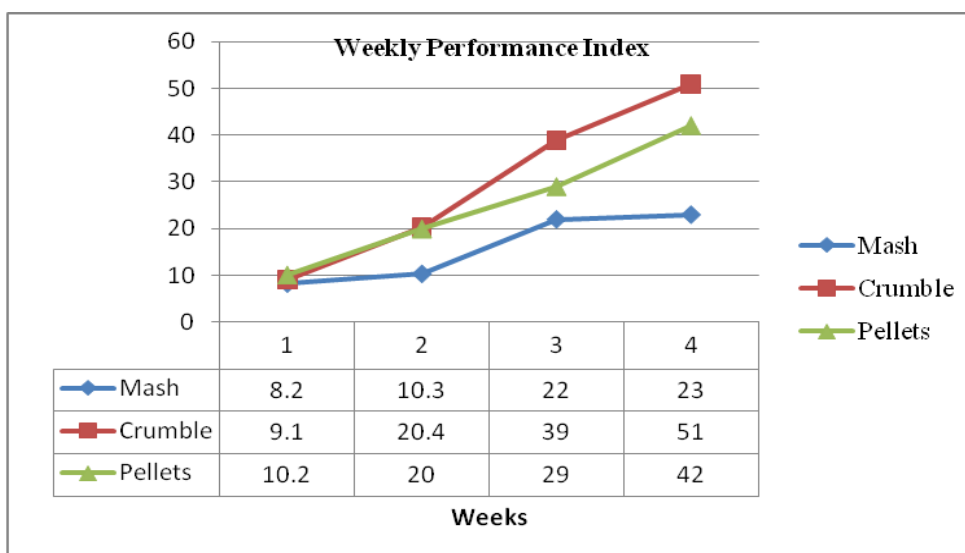


Figure 3 - Weekly performance index

Table 2 - Production and carcass parameters

Parameters	Feed Forms	Mash	Crumbles	Pellets	P - value ¹	CV ²
Feed intake (kg)		0.769±0.82 ^a	1.107±1.17 ^b	0.952±1.01 ^a	0.001	20.2
FCR ³ (kg/kg)		0.723±1.06	0.715±1.04	0.615±0.90	0.182	22.8
Average Live weight (kg)		1.090±0.74 ^a	1.655±1.13 ^b	1.663±1.13 ^b	0.013	34.6
Performance index		16.6±0.68 ^a	25.5±1.05 ^b	30.7±1.26 ^c	0.040	54.0
Abdominal fat Wt (g)		18.18±0.02 ^a	25.33±1.06 ^b	24±0.1.11 ^b	0.010	45.2
Carcass weight (kg)		1.052±0.68 ^a	1.845±1.19 ^b	1.751±1.13 ^b	0.001	8.0
Carcass yield (%)		71.67±4.17	74.97±0.89	73.21±2.63	0.777	6.9

^{a,b} Means in rows with different superscripts differ significantly (P≤0.05); ¹ (P≥0.05) = non significant; ² Coefficient of variation; ³Feed conversion ratio

Intestinal parameters

The effects of feed form on broiler gastrointestinal tract parameters results are shown in Table 3. According to these results dietary treatments had a significant (P≤0.05) effect on intestinal length while gizzard and intestinal weights were not statistically (P≥0.05) different between the dietary treatments. It was also observed that the increase in feed particle size from mash to pellets was negatively correlated with all intestinal parameters.

Table 3 - Intestinal parameters

Parameters	Feed Forms	Mash	Crumbles	Pellets	P - value ¹	CV ²
Gizzard Wt (g)		30.82±0.02	20.67±0.06	20.33±0.08	0.495	21.2
Intestinal Wt (g)		82.73±0.03	67±0.08	63.33±0.11	0.408	25.2
Intestinal Length (cm)		148.64±4.23 ^b	126.53±2.16 ^a	118.27±3.95 ^a	0.015	15.1

^{a,b} Means in rows with different superscripts differ significantly (p≤0.05); ¹ (p≥0.05) = non significant; ² Coefficient of variation

DISCUSSION

Production and carcass parameters

Feed intake and live weight results of the current study are in agreement with the findings of [Chewning et al. \(2012\)](#), [Chehraghi et al. \(2013\)](#), [Al-Nasrawi \(2016\)](#), [Gracia et al. \(2016\)](#) and [Omozebi et al. \(2016\)](#) who reported significantly (P≤0.05) higher body weight and feed intake in birds fed crumble and pelleted diets than birds fed mash diet. [Dozier et al. \(2010\)](#) working with Ross 708 male broiler chickens between day 15 and 42 reported similar results to the current study where they observed that different feed forms had no significant (P≥0.05) influence on carcass yield and FCR. The findings also concurred with the results of [Munt et al. \(1995\)](#), [Bolukbasi et al. \(2005\)](#), [Brickett et al. \(2007\)](#), [Agah and Norollahi \(2008\)](#), [Lv et al. \(2015\)](#) and [Ismail et al. \(2016\)](#) who studied the effect of feed form on broilers performance and reported that feed form did not have significant (P≥0.05) influence on FCR. Contrary to these results ([Quentin et al., 2004](#); [Jahan et al., 2006](#); [Salari et al., 2006](#); [Amerah et al., 2007](#) and [Agah and Norollahi, 2008](#)) found that different feed forms had significant (P≤0.05) influence on feed conversion ratios of broilers.

Observed carcass parameters results are in-line with the findings of [Nabi et al. \(2017\)](#) and [Hosseini and Afshar \(2017\)](#) who compared the effect of different feed form on carcass parameters and observed significant (P≤0.05) difference in carcass weight and abdominal fat between birds fed mash, crumble and pelleted diets. These researchers reported that birds fed mash diet had the lowest yield compared to other treatments. On the other hand [Mirghelenj and Golian \(2009\)](#) and [Dozier et al. \(2010\)](#) indicated that abdominal fat weight was not influenced by different feed forms. The contradicting results could have been due to differences in breeds and number of feeding phases used in different studies. [Dozier et al. \(2010\)](#) study used Ross 708 male broiler from day-old to day-42 while the current study used Ross 308 and covered growing and finishing phases between 16 and 42th day.

[Jensen et al. \(1962\)](#) and [McKinney and Teeter \(2003\)](#) revealed that pellet-fed birds spent less time in the act of consuming meals. It could be concluded that the increased growth rate of birds fed pelleted diets is accompanied by reduction in energy expenditure during meal consumption. Reduced energy expenditure would allow for an increase in productive energy (PE) value of the diet, thus providing more calories for protein & lipids synthesis in growing birds.

Intestinal parameters

The intestinal length results are in agreement with the results of Amerah et al. (2007) and Frikha et al. (2009) who reported significant ($P \leq 0.05$) longer intestinal length in birds fed mash diet. Amerah et al. (2007) also observed a reduction in the relative length of all GIT components as feed particle size increased. However, a decreased intestinal weight or length may result in improved feed efficiency due to reduced maintenance costs (Xu et al., 2015). Intestinal and gizzard weight results are in agreement with Amerah et al. (2007) and Senkoylu et al. (2009) and Abdollahi et al. (2011) and partly conform with results of Gabriel et al. (2008) and Mirghelenj and Golian (2009) who confirmed that pelleted diets reduced the weights of intestinal components. Zaefarian et al. (2016) confirmed that birds do not fully develop their upper gastrointestinal tract when highly processed pelleted feeds are used.

CONCLUSION

The findings of this current study revealed that feed forms for broiler resulted in significant impact in both production and carcass parameters during the whole experimental period, where birds fed crumble and pelleted diets performed better than birds fed diet in a mash form. With regard to gastrointestinal parameters it can be concluded that diet in the form of crumble and pellet form have a negative influence on the development of intestinal components such as gizzard and intestinal weight and length because they reduce the weight and length of these parameters.

Broiler diets in mash form despite its relatively cheaper cost resulted in significant lower production performance, low carcass yields and higher production of inedible parts (viscera). It is therefore recommended that farmers in Lesotho should opt for large particle size feed form such as crumble and pelleted diets if they want good broiler growth rates and high meat yields.

DECLARATIONS

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Authors' contribution

N.P.Kuleile contributed on design of experiment, data collection and the write up of the manuscript and S.M.Molapo participated in data analysis.

Conflict of interests

The authors have not declared any conflict of interests.

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Acknowledgements

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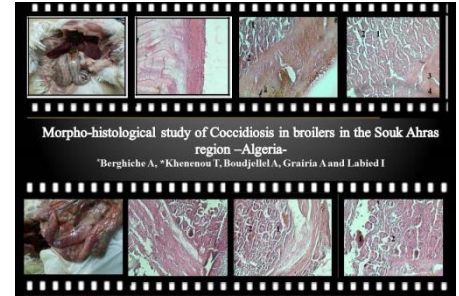
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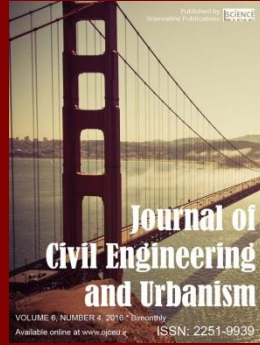
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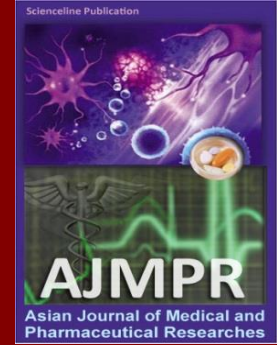
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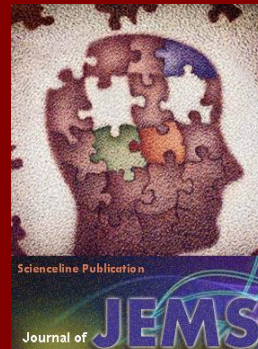
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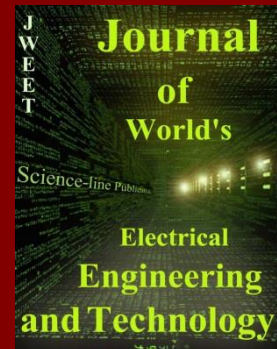
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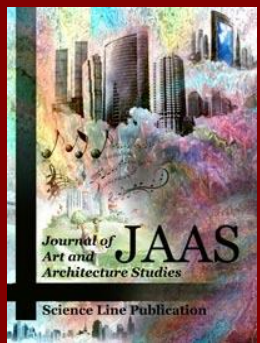
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