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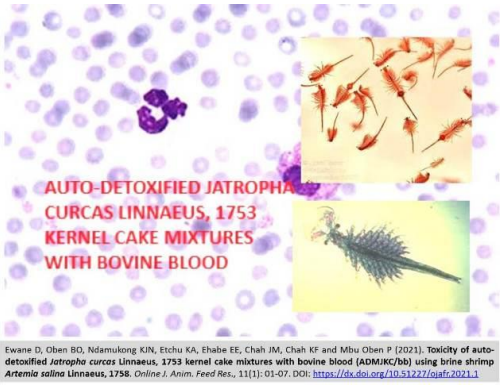
Research Paper

Toxicity of auto-detoxified *Jatropha curcas* Linnaeus, 1753 kernel cake mixtures with bovine blood (ADMJKC/bb) using brine shrimp *Artemia salina* Linnaeus, 1758.

Ewane D, Oben BO, Ndamukong KJN, Etchu KA, Ehabe EE, Chah JM, Chah KF and Mbu Oben P.

Online J. Anim. Feed Res., 11(1): 01-07, 2021; pii: S222877012100001-11
DOI: https://dx.doi.org/10.51227/ojafr.2021.1



Ewane D, Oben BO, Ndamukong KJN, Etchu KA, Ehabe EE, Chah JM, Chah KF and Mbu Oben P (2021). **Toxicity of auto-detoxified *Jatropha curcas* Linnaeus, 1753 kernel cake mixtures with bovine blood (ADMJKC/bb) using brine shrimp *Artemia salina* Linnaeus, 1758.** *Online J. Anim. Feed Res.*, 11(1): 01-07. DOI: https://dx.doi.org/10.51227/ojafr.2021.1

Abstract
In present study, brine shrimp (*Artemia salina* L.) was used to determine the toxicity of auto-detoxified Jatropha kernel cake (JKC) mixed with bovine blood (ADMJKC/bb). The powdered-JKC was mixed with bovine blood (bb) at three ratios (1:1=X, 2:1=Y and 3:1=Z of JKC: bb) and the resultant mixtures processed using four protocols: Heated, Spread Dry = 1, Unheated Spread Dry = 2, Heated Spread Remoisten to 66% dry matter (DM) = 3 and Unheated Spread Remoistened to 66% DM = 4). The resultant 12 treatment combinations (X1, X2, X3, X4, Y1, Y2, Y3, Y4, Z1, Z2, Z3 and Z4) were placed in a Solar *J. curcas* auto-detoxification apparatus from where samples were retrieved periodically and evaluated for detoxification using the brine shrimp lethality test. There were no significant differences within the same ratio of mixes among the four protocols. However, there was a tendency for mean LC_{50} values to increase between the ratios. Specifically, Protocol 2 recorded a significant difference between X2 and Z2 treatments, having 1:1 and 3:1 JKC: bb mixes respectively. Upon ranking the level of auto-detoxification, the most detoxified treatments (Z2 with LC_{50}=4674 and Z4 with LC_{50} =3692) differed significantly from the least two (X1 with LC_{50}=1383 and X2 with LC_{50}=1459). Addition of bovine blood to JKC increased the dynamics of JKC auto-detoxification, probably due to the presence of some innate auto-detoxifying microbial inoculum and bovine blood which boost the rapid growth, development and succession of these microbes. Thus combining JKC with bovine blood is complementary for JKC auto-detoxification, with the most detoxified ingredients (Z2, Z4 and Y3) appearing most suitable for further development and testing as feed ingredient for farm animals.
Keywords: Auto-detoxification, Bovine blood, Feedstuff, Jatropha, Shrimp.

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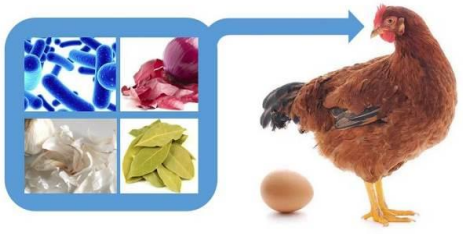
Research Paper

Effect of dietary supplementation of probiotic, phytobiotics or their combination on performance, blood indices and jejunal morphology of laying hens during post peak production.

Hidayat R, Yunianto VD, Sukamto B and Sugiharto S.

Online J. Anim. Feed Res., 11(1): 08-12, 2021; pii: S222877012100002-11
DOI: https://dx.doi.org/10.51227/ojafr.2021.2



Hidayat R, Yunianto VD, Sukamto B and Sugiharto S (2021). **Effect of dietary supplementation of probiotic, phytobiotics or their combination on performance, blood indices and jejunal morphology of laying hens during post peak production.** *Online J. Anim. Feed Res.*, 11(1): 08-12. DOI: https://dx.doi.org/10.51227/ojafr.2021.2

Abstract
The purpose of this study was to evaluate the effect of dietary supplementation of probiotic (*Lactobacillus acidophilus*), phytobiotics (bay leaves, onion peel and garlic peel) or their combination on blood parameters, morphology of digestive tract and performance of laying hens. The experiment used 144 laying hens aged 72 weeks old, and divided into 6 treatments including Ctl (basal feed as control); Pr (basal feed + 1.2 mL/day of probiotic *L. acidophilus*); Ph2 (basal diet + 2% phytobiotic of diet); PrPh2 (basal diet + 1.2 mL/day probiotic + 2% phytobiotic); PrPh4 (basal diet +1.2 mL/day probiotic + 4% phytobiotic) and PrPh6 (basal diet + 1.2 mL/day probiotic + 6% phytobiotic). Feed intake and egg mass were weekly recorded. One chick from each replicate was blood sampled and then slaughtered for data collection. Results showed that treatments had no effect on hemoglobin, erythrocyte and leukocyte of hens. The control hens had higher levels of cholesterol and LDL than that of PrPh2, PrPh4 and PrPh6 hens. HDL level tended to be higher in PrPh2 and PrPh4 as compared to control hens. Compared to control, the villi height of jejunum was higher in the treated hens, with PrPh4 had the highest villi height. Ileal protein digestibility tended to be higher in the treated than that in control hens. Also, there was a clear tendency that feed conversion ratio was lower in the treated hens than that of control. In conclusion, the combined use of probiotics and phytobiotics improved physiological condition, ileal histomorphology, ileal protein digestibility and feed conversion of laying hens during post peak production.
Keywords: Garlic, Herb, Laying Hen, Phytobiotic, Probiotic.

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Review

Efforts, successes and challenges of green feed production in Ethiopia.

Assefa Woldemariam G.

Online J. Anim. Feed Res., 11(1): 13-17, 2021; pii: S222877012100003-11
DOI: https://dx.doi.org/10.51227/ojafr.2021.3

Assefa Woldemariam G (2021). **Efforts, successes and challenges of green feed production in Ethiopia.** *Online J. Anim. Feed Res.*, 11(1): 13-17. DOI: https://dx.doi.org/10.51227/ojafr.2021.3

Abstract
Improving the feed supply and digestibility of roughage through green feed supplementation was the aim of forage production in Ethiopia. In spite of the time-consumed efforts made, the success is still low. This review summarizes efforts, challenges, good practices and indicates where the focus of future efforts should be. The efforts made in improve forage production (IFP) includes selection of forage species and development strategies, preparation of extension manual, training of stakeholders, formulation of forage seed production system, and introduction of hydroponic fodder. As a result farmers increased milk production and reduce feed cost by 20% and 40 %, respectively, and fatten ruminants in 2 to 3 cycles per year as a result of improved forage use. However, there is no management model for communal grazing land. IFP constrained by long dry period, scarcity of land, irrigation and the subsistence system of livestock production and the free grazing practice. Consequently, the communal grazing lands are devastatingly overstocked and degraded, livestock become dependent on crop residues, IFP practice remains low and livestock feed deficit is common. Therefore, it is recommended that future efforts should focus on designing communal grazing land tenure model and development package. Green forge production (GFP) should concentrate on livestock excluded areas and tree legumes to produce green feed in the dry period. GFP best practices should be expanded to the areas similar to that the practices found effective. Reduce feed deficit by improving crop production to increase the crop residue and the grain yield. The subsistence system of production should be transformed into market-oriented by introducing a technology package that reduces young stock mortality, increases fertility, calf/ lamb crop and livestock offtake rate. Technical simplicity and cost-effectiveness, of the hydroponic fodder system, should be assessed before implemented in a wider setting.
Keywords: Animal Nutrition, Forage, Grazing, Green feed, Livestock.

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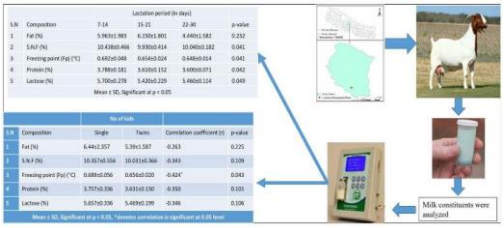
Research Paper

Effect of lactation length and litter size on milk composition of Boer goat within one month of parturition.

Regmi S, Mahato P and Sapkota KhR.

Online J. Anim. Feed Res., 11(1): 18-22, 2021; pii: S222877012100004-11
DOI: https://dx.doi.org/10.51227/ojafr.2021.4



Regmi S, Mahato P, Sapkota KhR (2021). **Effect of lactation length and litter size on milk composition of Boer goat within one month of parturition.** *Online J. Anim. Feed Res.*, 11(1): 18-22. DOI: https://dx.doi.org/10.51227/ojafr.2021.4

Abstract
The aim of this study was to determine the effect of lactation length and litter size on the milk composition of Boer goats. Milk samples from 23 lactating Boer goats reared in NLBO (National Livestock Breeding Office), Pokhara, Nepal, were collected and analyzed in a laboratory present within a farm. Goats were reared under the same environment and provided with similar care and management. Lactation length showed a non-significant effect on fat content in the milk whereas all other constituents were influenced significantly. Similarly, litter size showed a significant effect on the freezing point of milk only. Litter size and all milk constituents were negatively correlated. However, Litter size and freezing point showed moderately strong correlation. Twinning did not significant influence fat, protein, lactose and solids nonfat content of goat milk. All constituents were weakly correlated to litter size however freezing point showed moderately strong correlation. Thus, more focus must be given to the nutritional management of triplets and twins to improve their growth rate compared to a single kid.
Keywords: Boer goat, Lactation length, Milk composition, Litter size, Twinning.

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Research Paper

Characteristics of ostrich meat in manufacturing sausage in comparison with beef.

Elhashmi YH, Falih SY and Abdalmageed MEI.

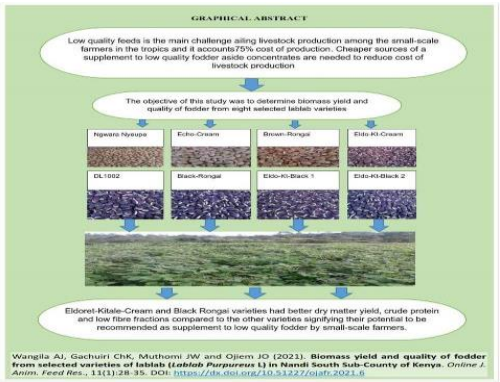
Online J. Anim. Feed Res., 11(1): 23-27, 2021; pii: S222877012100005-11
DOI: https://dx.doi.org/10.51227/ojafr.2021.5

Elhashmi YH, Falih SY and Abdalmageed MEI (2021). **Characteristics of ostrich meat in manufacturing sausage in comparison with beef.** *Online J. Anim. Feed Res.*, 11(1): 23-27. DOI: https://dx.doi.org/10.51227/ojafr.2021.5

Abstract

Ostrich meat has become one of the most popular meat around the world. The objectives of this study are to evaluate the quality characteristics of processed meat, compared to products made from beef. The Ostrich and beef meat was prepared and stored frozen. Sausage was processed in laboratory and analyzed for chemical composition (crude protein, moisture, fat and ash). Physical properties include Water-holding capacity (WHC), cooking loss and microbiological analysis were done. The findings of this study revealed that, chemical composition of sausage from ostrich and beef had highly significant differences in moisture, crude protein, lipids and ash among all types of products. Physical attribute of sausage revealed that, there were highly significant differences among all types of products. The microbiological in sausage observed that, the highly significant differences in *E. coli*, *staphylococcus aurous*, total coli form, yeast and mold and total viable count but was not significant differences in Pseudomonas among all types of sausage. The study recommended that ostrich meat can be used on manufacturing of meat as an alternative to beef meat in limited amount.
Keywords: Animal Product, Meat, Ostrich, Processing, Sausage.

Research Paper



Biomass yield and quality of fodder from selected varieties of lablab (*Lablab Purpureus* L) in Nandi South Sub-County of Kenya.

Wangila AJ, Gachuiri ChK, Muthomi JW and Ojiem JO.

Online J. Anim. Feed Res., 11(1): 28-35, 2021; pii: S222877012100006-11
DOI: https://dx.doi.org/10.51227/ojafr.2021.6

Abstract
Low quality feeds is the main challenge ailing livestock production among the small-scale farmers in the tropics. Cheaper sources of alternative high quality fodder supplements are needed to improve livestock productivity. The objective of this study was to determine biomass yield and quality of fodder from selected lablab varieties. Eight lablab varieties namely, DL1002, Ngwara Nyeupe, Echo-Cream, Black-Rongai, Eldo-Kt-Cream, Eldo-Kt-Black1, Brown Rongai and Eldo-Kt-Black2 were established in three sites of Nandi south sub county, Kenya. Randomized complete block design was used at farm level with four replications per site. Data on biomass yield, chemical composition and in vitro-dry matter digestibility of the eight lablab forages was collected. Biomass yield differed significantly among the lablab varieties ranging from 5.6-12.6 t DM/ha across the three sites. Highest biomass yield was recorded for Brown Rongai (12.6 t DM/ha) and lowest with DL1002 (5.6 t DM/ha). Crude protein (CP) content varied significantly between varieties with sites ranging from 19.6-23.9 g/100g. Highest CP was recorded with Eldo-Kt-Cream and Black Rongai (23.9 g/100g and 23.7 g/100g) across the three sites. For all the varieties, Neutral detergent fibre (NDF) ranged from 44.4-48.6 g/100g, acid detergent fibre (ADF) 31.6-35.7 g/100g and acid detergent lignin (ADL) 9.0-11.9 g/100g across the three sites. Highest NDF was recorded with DL1002 (48.6 g/100g), ADF with Eldoret-Kitale-Black2 (35.9 g/100g) and acid detergent lignin with DL1002 (11.7 g/100g). In vitro dry matter digestibility (IVDMD) varied significantly between varieties and sites ranging from 67.6-75.7 g/100g between the varieties across the three sites. Eldo-Kt-cream and Black Rongai had the highest IVDMD (75.7 and 74.4 g/100g) across the three sites. Eldoret-Kitale-Cream and Black Rongai varieties had better dry matter yield, crude protein and low fibre fractions compared to the other varieties signifying their potential to be recommended as supplement to low quality fodder by small-scale farmers.
Keywords: Biomass yield, Digestibility, Feed, Fodder, Lablab.

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TOXICITY OF AUTO-DETOXIFIED *Jatropha curcas* Linnaeus, 1753 KERNEL CAKE MIXTURES WITH BOVINE BLOOD (ADMJKC/BB) USING BRINE SHRIMP *Artemia salina* Linnaeus, 1758

Divine EWANE[1], Benedicta O. OBEN[2], Kenneth Jacob Ngoh NDAMUKONG[1], Kingsley A. ETCHU[3], Eugene E. EHABE[3], Jane M. CHAH[4], Kennedy F. CHAH[5] and Pius MBU OBEN[2]

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Supporting Information

RESEARCH ARTICLE

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ABSTRACT: In present study, brine shrimp (*Artemia salina* L.) was used to determine the toxicity of auto-detoxified Jatropha kernel cake (JKC) mixed with bovine blood (ADMJKC/bb). The powdered-JKC was mixed with bovine blood (bb) at three ratios (1:1=X, 2:1=Y and 3:1=Z of JKC: bb) and the resultant mixtures processed using four protocols: Heated, Spread Dry = 1, Unheated Spread Dry = 2, Heated Spread Remoisten to 66% dry matter (DM) = 3 and Unheated Spread Remoistened to 66% DM = 4). The resultant 12 treatment combinations (X1, X2, X3, X4, Y1, Y2, Y3, Y4, Z1, Z2, Z3 and Z4) were placed in a Solar *J. curcas* auto-detoxification apparatus from where samples were retrieved periodically and evaluated for detoxification using the brine shrimp lethality test. There were no significant differences within the same ratio of mixes among the four protocols. However, there was a tendency for mean LC_{50} values to increase between the ratios. Specifically, Protocol 2 recorded a significant difference between X2 and Z2 treatments, having 1:1 and 3:1 JKC: bb mixes respectively. Upon ranking the level of auto-detoxification, the most detoxified treatments (Z2 with LC_{50}=4674 and Z4 with LC_{50} =3692) differed significantly from the least two (X1 with LC_{50}=1383 and X2 with LC_{50}=1459). Addition of bovine blood to JKC increased the dynamics of JKC auto-detoxification, probably due to the presence of some innate auto-detoxifying microbial inoculum and bovine blood which boost the rapid growth, development and succession of these microbes. Thus combining JKC with bovine blood is complementary for JKC auto-detoxification, with the most detoxified ingredients (Z2, Z4 and Y3) appearing most suitable for further development and testing as feed ingredient for farm animals.

Keywords: Auto-detoxification, Bovine blood, Feedstuff, Jatropha, Shrimp.

Abbreviations: ADJKC; auto-detoxified Jatropha kernel cake; ADMJKC/bb: auto-detoxified mixtures Jatropha kernel cake and bovine blood; ANOVA: analysis of variance; BSLT: Brine Shrimp Lethality Test; bb: bovine blood; CMJKC/bbE: crude methanol auto-detoxified mixtures of Jatropha kernel cake with bovine blood extracts; DJADA: Diffuse daylight *Jatropha curcas* auto-detoxification apparatus; DMSO: dimethyl sulfoxide; DMRT: Duncan's Multiple Range Test; FAO: Food and Agriculture Organization of the United Nations; IBM: International Business Machines; JKC: Jatropha kernel cake; LC_{50}: Lethal concentration killing 50% of test organisms; NRC: National Research Council; SJADA: Solar *Jatropha curcas* auto-detoxification apparatus; SPSS: Statistical package for social sciences; UDC: un-moistened diffuse daylight spread; USS: un-moistened solar spread; X1: Jatropha kernel cake and bovine blood, mixed at a ratio of 1:1. Heated, spread dried without remoistening; X2: Jatropha kernel cake and bovine blood, mixed at a ratio of 1:1. Unheated, spread dried without remoistening; X3: Jatropha kernel cake and bovine blood, mixed at a ratio of 1:1. Heated, spread dried, remoisten to 66% dry matter; X4: Jatropha kernel cake and bovine blood, mixed at a ratio of 1:1. Unheated, spread dried, Remoistened to 66% dry matter; Y1: Jatropha kernel cake and bovine blood, mixed at a ratio of 2:1. Heated, spread dried without remoistening; Y2: Jatropha kernel cake and bovine blood, mixed at a ratio of 2:1. Unheated, spread dried without remoistening; Y3: Jatropha kernel cake and bovine blood, mixed at a ratio of 2:1. Heated, spread dried, remoisten to 66% dry matter; Y4: Jatropha kernel cake and bovine blood, mixed at a ratio of 2:1. Unheated, spread dried, remoistened to 66% dry matter; Z1: Jatropha kernel cake and bovine blood, mixed at a ratio of 3:1. Heated, spread dried without remoistening; Z2: Jatropha kernel cake and bovine blood, mixed at a ratio of 3:1. Unheated, spread dried without remoistening; Z3: Jatropha kernel cake and bovine blood, mixed at a ratio of 3:1. Heated, spread dried, remoisten to 66% dry matter; Z4: Jatropha kernel cake and bovine blood, mixed at a ratio of 3:1. Unheated, spread dried, Remoistened to 66% dry matter.

INTRODUCTION

Jatropha curcas has shown some promise as a crop from which biofuels can be produced without compromising human food needs (Nithiyanantham et al., 2012; Maghuly and Laimer, 2013). However, despite global excitement on its potentials, at current levels of technology, value-addition from its by-products and co-products is necessary in order for viability of the sector to be ensured (Makkar et al., 2012). One of the by-products being evaluated in this regard is Jatropha kernel cake (JKC), the remnants obtained after extraction of oil from Jatropha kernels that have a potential for use as animal feed (Nithiyanantham et al., 2012; Che Hamzah et al., 2020). Except for lysine, its nutrient profile surpasses the Food and Agriculture Organization of the United Nations (FAO)'s reference protein (Makkar et al., 1998).

Citation: Ewane D, Oben BO, Ndamukong KJN, Etchu KA, Ehabe EE, Chah JM, Chah KF and Mbu Oben P (2021). Toxicity of auto-detoxified *Jatropha curcas* Linnaeus, 1753 kernel cake mixtures with bovine blood (ADMJKC/bb) using brine shrimp *Artemia salina* Linnaeus, 1758. *Online J. Anim. Feed Res.*, 11(1): 01-07. DOI: https://dx.doi.org/10.51227/ojafr.2021.1

However, it is toxic to humans and animals because of its phorbol esters and other anti- nutrients (trypsin inhibitors, lectins and phytate) and therefore requires detoxification prior to use (Sharath et al., 2014; Rodríguez-González et al., 2018).

Several methods targeting the larger industries and Jatropha farm holdings have been employed and categorised with varying degrees of success to detoxify JKC (Ewane et al., 2017). However, the need for simple cottage techniques for small and large- scale operations persists. Auto-detoxification of JKC has therefore been developed as a pro-poor and pro-rural detoxification method for JKC (Ewane et al., 2017).

Present study aimed to determine the toxicity of auto-detoxified mixtures JKC and bovine blood (ADMJKC/bb) on brine shrimp (*Artemia salina* L.). The strategy was to manipulate endogenous and environmental factors that can enhance self-detoxification in mixtures of JKC and bovine blood and identify the most promising treatment combinations. Bovine blood, often used to produce blood meal, highly supports microbial growth, and also represents one of the richest sources of lysine (NRC, 1994). Hence, the (ADMJKC/bb) mix shall enhance the nutrient profile and detoxification rate of JKC. Furthermore, the powdery ground JKC shall easily trap the liquid bovine blood and reduce the need for further processing of blood meal by small-scale farmers (Nithiyanantham and Francis, 2012).

MATERIALS AND METHODS

Study Location

The study used the facilities of the Teaching and Research Farm, University of Buea, Cameroon (located at 4.1667^0 N, 9.2333^0 E, 578 m asl). *J. curcas* seeds were harvested from farms, live fences and plantations in four of Cameroon's five agro-ecological zones (Figure 1): Maroua in the Sudano-Sahel zone, Ngaoundere in the High Guinean Savana, Bamenda in the Western Highlands and Mamfe in the Humid Forest zone with a mono-modal rainfall pattern. Bovine blood was collected from the main abattoir in the town of Buea.

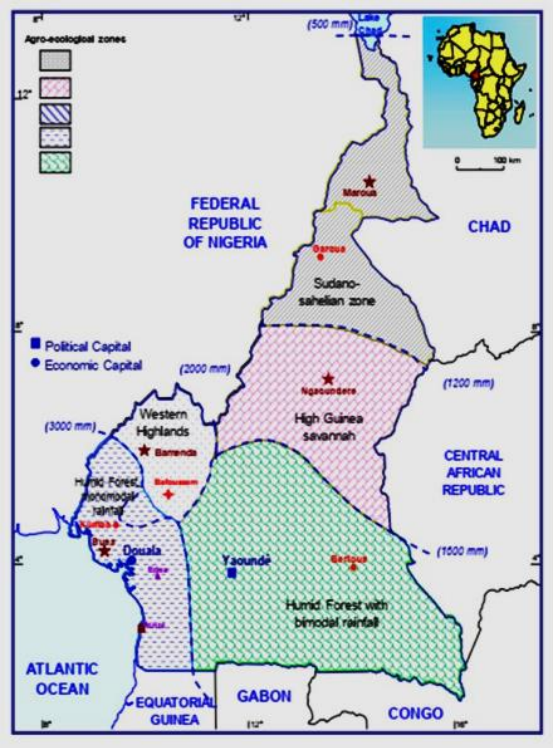

Figure 1 - Location map of Cameroon showing the agro-ecological zones where *J. curcas* seeds were collected.

Preparation and pre-treatment of *J. curcas* kernel cake and bovine blood mix

J. curcas seeds were cracked open (using two hard boards) and the extracted kernels de-oiled using a hydraulic press. The *J. curcas* kernel cake (JKC) obtained was then finely ground using a plate mill and the powder homogenized by hand mixing before further mixing with bovine blood in three different proportions (1/1, 2/1 and 3/1).

Twelve combinations were developed for production and evaluation based on four processing protocols of JKC mixtures with bovine blood (bb) at three mix ratios (Table 1). The mixes were chosen by serially increasing the quantity of JKC in a fixed amount of bovine blood. This gave combination protocols from three ratio mixes and four auto-detoxification treatments. These protocols were inspired by previous studies on the effects of moisture (Abou-Arab and Abu-Salem, 2010), spreading (Schmidt and Hecker, 1975) and heat (Aregheore et al., 2003; Martinez-Herrera et al., 2006) on degradation of phobol esters and/or associated anti- nutrients of JKC.

Each combination was replicated 4 times and placed in steel plates arranged on two tables within the performant SJADA (Ewane et al., 2017), operated at full detoxification mode with the air access inlet closed to the minimum level of 50 cm^2. Six of the 12 treatment combinations (Protocol code 3 and code 4: X3, Y3, Z3, X4, Y4 and Z4) were remoistened to 66 % dry matter daily, while the other six (Protocol code 1 and code2: X1, Y1, Z1, X2, Y2 and Z2) were not remoistened. About 10 g of sample were collected weekly from each replicate to evaluate the level of auto-detoxification

Table 1 - Treatment codes of JKC/bb mixing ratios, auto-detoxification and moistening cycles.

Protocol Code	Auto-detoxification treatment of JKC: bb combination Protocol	JKC: bb mixing ratio		
		X (1:1)	Y (2:1)	Z (3:1)
1	Heat, spread and dried - No remoistening	X1	Y1	Z1
3	Heat, spread and dried - Remoistened to 66% dry matter	X3	Y3	Z3
2	No heat, spread and dried - No remoistening	X2	Y2	Z2
4	No heat, spread and dried - Remoistened to 66% dry matter	X4	Y4	Z4

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Preparation of crude methanol auto-detoxified mixtures of JKC/bovine blood extracts (CMJKC/bbE)

A sample of about 40 g (10 g per replicate) was collected weekly from each treatment and analysed to determine the progress of auto-detoxification. The replicates of each treatment were pooled and further dried within the apparatus for another week, homogenised and powdered. From the powdered mass, 20 g were placed in an extraction bottle containing 200 mL methanol and the methanolic extract obtained by regularly stirring the whole for 72 h using a rotatory stirrer. The extract was then filtered through Whatman No. 541 filter papers and the crude methanol auto-detoxified mixtures of JKC/bovine blood extracts (CMJKC/bbE) obtained by evaporation of the solvent using a rotary evaporator.

Brine shrimp bioassay

The brine shrimp lethality test (Meyer et al., 1982) was used, with some modifications, to test the toxicity of CMJKC/bbE. Brine shrimps (*Artemia salina* Leach) were hatched from eggs in rectangular dishes containing sea water. The dishes were sub-divided by a perforated barrier into two unequal chambers and kept under constant aeration. The bigger chamber receiving the eggs was in the dark while the smaller chamber receiving the hatched napuli in anticipation was under constant light. Forty-eight hours was allowed for the eggs to hatch and the phototropic napuli to mature. At each turn, 10 napuli were drawn using a pipette and placed in a marked vial containing 4 ml of natural seawater. Prior to this, a CMJKC/bbE stock solution of 100,000 ppm was prepared by dissolving 200 mg of CMJKC/bbE in 2 ml of dimethyl sulfoxide (DMSO). The stock solution was then diluted serially with natural seawater to give a series of concentrations for testing (10 000, 1000, 100, 10 and 1 ppm). Three replicates of each concentration were prepared. From each pre-dilution, 1 ml containing CMJKC/bbE, seawater and DMSO was added to the pre-marked vials containing 4 ml of natural sea water and 10 napuli to make a total volume of 5 ml. Negative controls were just dilutions of DMSO in seawater without CMJKC/bbE while un-detoxified whole Jatropha kernel cake served as the positive control.

The vials were incubated under light for 24 h, after which manual counting of dead and immobile napuli at bottom of vial commenced, against a lit background with the aid of a 3X magnifying hand lens. The mortality was calculated as the percentage ratio of the number of dead napuli to the total number of napuli tested after corrections to account for mortalities recorded in the control (Abbott, 1925) as shown in equation 1. Subsequently corrections were made for 0% and 100% as proposed by Ghosh (1984) and presented in equation 2 and equation 3, respectively.

$$Corrected\ mortality\ (\%) = \{(M_{obs} - M_{con})/(100 - M_{con})\}x100 \quad \ldots\ldots \quad \text{Eqn. 1}$$

Where,

M_{obs} and M_{con} were the respective observed and control mortalities.

$$0\%\ Corrected\ mortality\ (\%) = 100\ x\ (0.25\ x\ n) \quad \ldots\ldots \quad \text{Eqn. 2}$$

$$100\ \%\ Corrected\ mortality\ (\%) = 100\ x\ (n - 0.25/n) \quad \ldots\ldots \quad \text{Eqn. 3}$$

Where,

n is the number of test animals in each group.

Determination of lethal concentration

The lethal concentration of CMJKC/bbE resulting in 50 % mortality of brine shrimp (LC_{50}) was determined from the 24 h counts by a plot of percentage of the shrimps killed against the logarithm of the CMJKC/bbE concentration and the best-fit line was obtained from the curve data by means of regression analysis (MS Excel version 7). The LC_{50} was derived from the slope of the best-fit line obtained.

Statistical analysis

Levene's Test for Equality of Variances was performed on LC_{50}. Also LC_{50} values were subjected to a one-way analysis of variance (ANOVA) and the significance of the differences between means tested using Duncan's Multiple Range Test (DMRT) ($P<0.05$). The software used was the IBM SPSS Statistics version 22 (IBM Corp. Released 2013). The most promising ADMJKC/bb treatments were selected after ranking the LC_{50} from the largest to the smallest values, with the largest values indicating the least toxicity. ANOVA and DMRT were used to detect significant difference of the LC_{50} values of ADMJKC/bb treatments

RESULTS

The evolution of LC_{50} of crude methanol auto-detoxified mixtures of JKC/bovine blood extracts (CMJKC/bbE) with duration of incubation have been presented in Figure 2, while: comparison of 3 week means of LC_{50} of crude methanol auto-detoxified mixtures of JKC/bovine blood extracts (CMJKC/bbE) is in Table 2. By the third week of the trial, all the 12 treatments had LC_{50} values above 1000. This indicates they were all substantially detoxified. There were no significant differences ($P>0.05$) among the four Auto-detoxification treatment of JKC: bb combination protocols when compared within rations for each ratio of mixes.

However, there was a general tendency for mean LC_{50} values to increase across ratios as quantity of JKC increased in the mixture. The only exception was Protocol code3 (the Heat, spread and dried - Remoistened to 66% dry matter

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protocol). In Protocol code 3, the 2:1 ratio (Y3) is higher than both the 1:1ratio (X3) and 1:3 ratios (Z3). In addition, Protocol code2 (the no heat, spread and dried - no remoistening protocol) recorded a significant difference ($P<0.05$) between X2 and Z2 treatments which are compared across ratios.

The ranking of mean LC_{50} (Table 3) indicates that the most detoxified treatments were Z2=3,047.22, Z4=2, 0130.67 and Y3=1,997.64, while the least detoxified were X2=653.65 X1=736.96 and Y1= 1,092.93. The top two most detoxified treatments (Z2 and Z4) differed significantly ($P<0.05$) from the least two (X1 and X2).

Of all the treatments evaluated, the extent of lethality was found to be proportional to the concentration of the CMJKCE/bbE. High mortalities were recorded at 1000 ppm and 10,000 ppm, while lower mortalities were recorded at 1 ppm and 10 ppm. The graphical representations of the corrected brine shrimp mortalities in week 3 for the most and least detoxified treatments as well as the un treated whole Jatropha kernel cake control are shown in Figure 3.

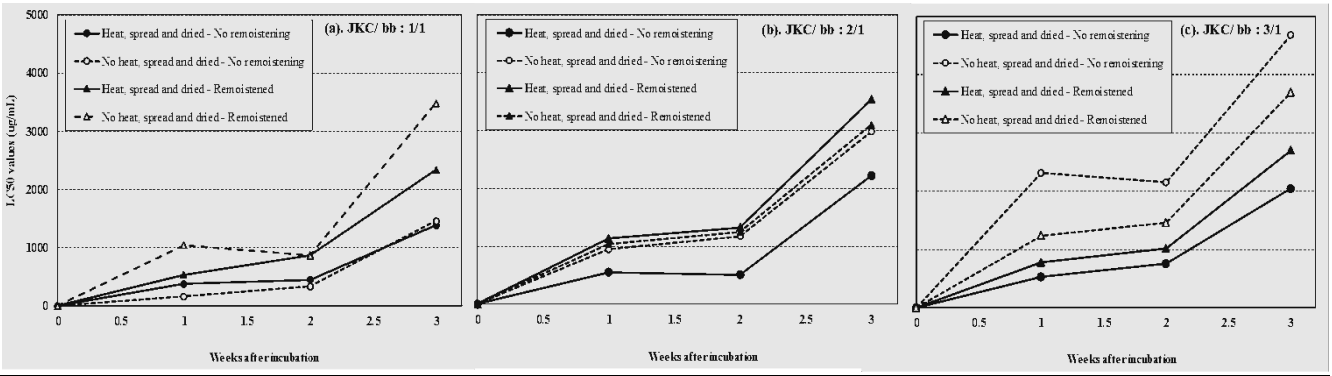

Figure 2 - Evolution of LC_{50} of crude methanolic auto-detoxified mixtures of JKC/bovine blood extracts (CMJKC/bbE) with duration of incubation

Table 2 - Comparison, LC_{50} of crude methanol auto-detoxified mixtures of JKC/bovine blood extracts (CMJKC/bbE) mean of 3 weeks

Protocol Code	Auto-detoxification treatment of JKC: bb combination Protocol	JKC: bb mixing ratio		
		X (1:1)	Y (2:1)	Z (3:1)
1	Heat, spread an d dried - No remoistening	X1 737.0 ± 844.7 [a]	Y1 1092.93 ± 783.83 [ab]	Z1 1110.46 ± 652.12 [ab]
2	No heat, spread and dried - No remoistening	X2 653.7 ± 604.5 [a]	Y2 1700.64 ± 771.72 [ab]	Z2 3047.22 ± 553.13 [b]
3	Heat, spread and dried - Remoistened to 66% dry matter	X3 1248.5 ± 815.2 [ab]	Y3 1997.64 ± 643.05 [ab]	Z3 1498.18 ± 406.18 [ab]
4	No heat, spread and dried - Remoistened to 66% dry matter	X4 1796.5 ± 323.7 [ab]	Y4 1794.53 ± 470.58 [ab]	Z4 2130.67 ± 563.01 [b]

[a,b,c:] Mean LC_{50} values with different superscript differ significantly ($P<0.05$).

Table 3 - Ranking of LC_{50} of crude methanol auto-detoxified mixtures of JKC/bovine blood extracts (CMJKC/bbE)

Treatment	Mean (± sem) LC_{50}	Group	RANK	Best fit equation	R^2
Z2	3047.22 ± 553.13	b	1	y = 22.568x - 32.795	0.8502
Z4	2130.67 ± 563.01	b	2		
Y3	1997.64 ± 643.05	ab	3	y = 23.337x - 32.795	0.8941
X4	1796.54 ± 323.73	ab	4		
Y4	1794.53 ± 470.58	ab	5		
Y2	1700.64 ± 771.72	ab	6		
Z3	1498.18 ± 406.18	ab	7		
X3	1248.46 ± 815.19	ab	8		
Z1	1110.46 ± 652.12	ab	9		
Y1	1092.93 ± 783.83	ab	10		
X1	736.96 ± 844.73	a	11	y = 21.415x - 21.645	0.8254
X2	653.65 ± 604.48	a	12	y = 21.799x - 18.954	0.9534
JS Control	0.05			y = 8.7287x + 61.229	0.6719

Values are means ± standard deviations of results obtained after 3 weeks. [a,b,c:] Mean LC_{50} values with different superscript differ significantly ($P<0.05$).

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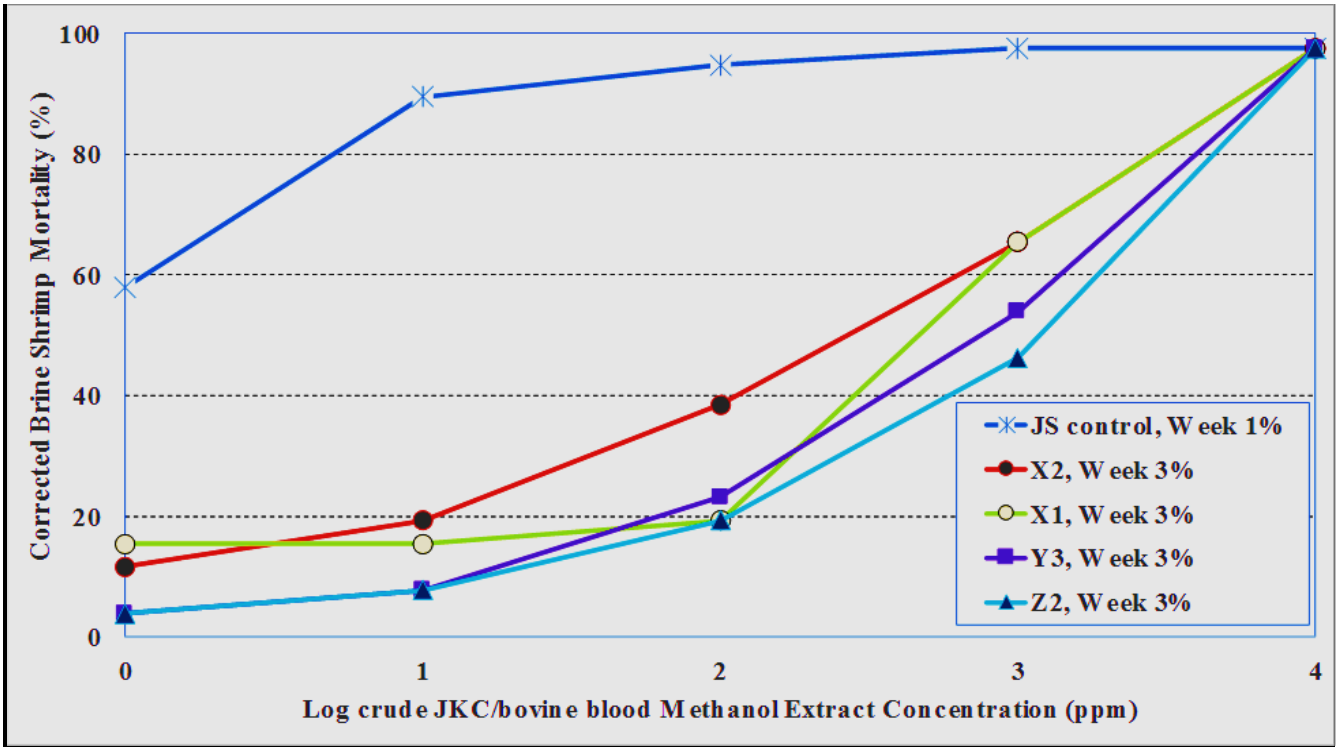

Figure 3 - Corrected brine shrimp mortality rates for different protocols and mixtures of Jatropha kernel subjected to different levels of auto-detoxification

DISCUSSION

According to Meyer et al. (1982), several extracts derived from natural products which had $LC_{50} \leq 1000$ µg/ml using Brine Shrimp Lethality Test (BSLT) were known to contain physiologically active principles while those with LC_{50} values > 1000 ppm were considered inactive. This indicates that all the 12 ADMJKC/bb ingredients were substantially detoxified after 3 weeks of exposure in the SJADA. The evolution of the LC_{50} values indicates that detoxification was more rapid with increasing content of JKC in the ADMJKC/bb ingredients. Two ingredients containing a ratio of 3:1 JKC: bb (Z2 and Z4) and two containing JKC: bb ratio of 2:1 (Y3 and Y4) all had LC_{50} values above 1000 within the first week of auto detoxification compared to just one ingredient containing a ratio of 1:1 JKC: bb (X4). Nonetheless, this level of auto-detoxification for even the least ratio mix of JKC with bovine blood is remarkably rapid, when compared to LC_{50} values of auto-detoxified JKC (ADJKC) ingredients produced solo without addition of bovine blood as reported in Ewane et al. (2017). In that study which was performed under similar conditions as the current study, it took 4 weeks for just 3 out of 14 ADJKC test ingredients on trial, to attain LC_{50} values above 1000.

Therefore, the addition of bovine blood to JKC probably changed the dynamics of auto- detoxification. According to Thomas (1988), in the live animal blood is generally a sterile medium, possessing innate bacteriostatic and bactericidal abilities. Such antibacterial effects are clearer with gram negative bacteria such as *E-coli*, and non-virulent strains of *Vibro cholerae, Haemophilus influenae,* salmonellae and shigellae. Out of the animal, however, bovine blood is a rich nutrient medium encouraging the growth of several microbes. Adding bovine blood to JKC may therefore increase the range of microbes in the mixture which would rapidly increase the level of auto-detoxification. Kasuya et al. (2013) increased the level of bio- detoxification of JSC by adding 10% eucalyptus bark. From the data obtained, they deduced that the importance of adding eucalyptus bark served to balance carbon and nitrogen and decrease the fat content, thus resulting in improved fungal growth. They concluded that their results support the hypothesis that phorbol ester degradation occurs because of co-metabolism by the enzymes responsible for lignin de-polymerization.

Even though the differences were not significant ($P>0.05$), ADMJKC/bb produced by subsequent remoistening irrespective of whether they were preheated or not (Protocol code 3 and code 4: Z3, Y3, X3, Z4, Y4, X4) as a group were among the top most detoxified, while the ADMJKC/bb preheated without subsequent remoistening (Protocol code 1: X1, Y1 and Z1) were the least detoxified. Interestingly, the actual most detoxified, median most detoxified and actual least detoxified ADMJKC/bb ingredients were respectively Z2, Y2 and X2, from protocol code 2. These ingredients were unheated and un-remoistened; the difference between the three ingredients was the ratio of mixing JKC with bovine blood. They were respectively mixed at 3:1, 2:1 and 1:1 for Z2, Y2 and X2. The protocol code 1 (Z1, Y1, and X1) ADMJKC/bb ingredients which were the least detoxified as a group followed a similar detoxification ranking with Z1 as the most detoxified and X1 as the least detoxified for that group. What was common among the Protocol code 1 and code 2: Z1, Y1, X1 Z2, Y2, X2 groups was that they were all undergoing a form of unperturbed solid state auto-detoxification compared to their daily remoistened counterparts of Protocol code 3 and code 4 (Z3, Y3, X3 & Z4, Y4, X4). The only

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difference is that while the Protocol Code 1 (Z1, Y1, X1) group was heated, the Protocol code 2 (Z2, Y2, X2) group was unheated. Similarly, while the Protocol code 3 (Z3, Y3, X3) group was preheated, the protocol code 4 (Z4, Y4, X4) group was unheated.

The differences observed among the various ratio mixes of JKC with blood therefore, probably indicate that the higher the ratio of JKC in the mixture, the higher the level of innate auto-detoxifying microbial inoculum. The hypothesis that needs to be verified is that "Microbes supporting auto-detoxification are present on the JKC and either act or wait for their opportunity to act in an ecological succession. Bovine blood provides a trigger (probably nutrients in simple molecules plus additional microbes) which boosts the rapid growth, development and succession of these microbes". Evidence from an earlier study by Ewane et al. (2017) in support of this hypothesis is that powdered JKC treatments with neither addition of water nor lye (un-moistened solar spread-USS- and un-moistened diffuse daylight spread-UDC-) treatments were substantially detoxified, having LC_{50} of 63.89 and 5.22 respectively after four weeks of exposure in the Solar *J. curcas* Auto-detoxification Apparatus (SJADA) and Diffuse Daylight *J. curcas* Auto-detoxification Apparatus (DJADA) respectively. The observation that the ADMJKC/bb preheated without subsequent remoistening (Protocol code 1: X1, Y1 and Z1) as a group were the least detoxified is a pointer that the initial inoculum was probably destroyed by heat and a succession was slower to take off in the absence of added moisture. The abnormal higher ranking of Y3 over Z3 in Protcol code 3 is possibly an indication that release of nutrients and other factors from bovine blood as a proportion to available microbial inoculum to auto-detoxifying microbes in heat treated and subsequently remoistened ADMJKC/bb could also be a determinant to the rate of auto- detoxification.

Furthermore, the results of Chikpah and Demuyakor (2012), who observed that approximately 60% reduction in crude phorbol ester levels can be achieved within 21 days of spontaneous fermentation of *J. curcas* kernel meal, supports the hypothesis that JKC contains some innate auto-detoxifying microbial inoculum. As defined in Ewane et al. (2017), auto-detoxification is a self-detoxification process induced by endogenous and environmental factors including enzymes, microbes, sunlight, temperature, humidity and wind. It is the natural way to transform the toxic *J. curcas* seeds into an innocuous material. These processes take considerable time under natural conditions but their duration can be shortened by human manipulation. The results of this present study therefore highlight the possible role of microbes in the auto-detoxification process. Working with pure microbial cultures, some authors including Belewu and Akande (2010), Belewu et al. (2010), Bose and Keharia (2013), Kasuya et al. (2013) and Azhar et al. (2014) have confirmed the role of fungi in the detoxification of JKC, while others including El-Zelaky et al. (2011), Phengnuam and Suntornsuk (2013), Widiyastuti et al. (2013) and Chang et al. (2014) have confirmed the role of bacteria in the detoxification of JKC.

All the 12 ADMJKC/bb ingredients were substantially detoxified; however, the top three most detoxified ingredients (Z2, Z4 and Y3) have been selected for further development. Any of the bottom nine (X4, Y4,Y2, Z3,X3,Z1,Y1,X1, and X2) may still be tested with animal models for further differentiation.

CONCLUSION

The addition of bovine blood to Jatropha Kernel Cake (JKC) changed the dynamics of auto-detoxification. Microbes supporting auto- detoxification are present on the JKC and either act or wait for their opportunity to act in an ecological succession. Bovine blood provides a trigger (probably nutrients in simple molecules plus additional microbes) which boosts the rapid growth development and succession of these microbes. Also, blood, which is the richest natural source of lysine, could supplement the reported lysine deficiency in JKC and the combined auto-detoxified mixtures Jatropha kernel cake and bovine blood; (ADMJKC/bb), would potentially become a better feed ingredient than either blood meal alone or JKC alone. Consequently, the top three most detoxified ingredients (Z2, Z4 and Y3) have been selected for further development and testing as feed ingredient for farm animals. However any of the bottom nine (X4, Y4,Y2, Z3,X3,Z1,Y1,X1, and X2) may still be tested with animal models for further differentiation.

DECLARARATIONS

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Authors' contribution:

D.Ewane conceived the study, designed the study, collected data, contributed in data analysis, and writing the manuscript, coordinated the inputs of all the other authors; B.O.Oben and K.J.N.Ndamukong performed critical reviewing of the manuscript and supervision of the study; K.A.Etchu performed critical reviewing of the manuscript; E.E.Ehabe contributed in design of study, data analysis and writing the manuscript; J.M.Chah contributed in data collection and write up of the manuscript; K.F.Chah contributed in conception of the study, design of study, data analysis and critical reviewing of the manuscript; P.M.Oben performed critical reviewing of manuscript and coordinated the study.

Competing interest

The authors have not declared any conflict of interest

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EFFECT OF DIETARY SUPPLEMENTATION OF PROBIOTIC, PHYTOBIOTICS OR THEIR COMBINATION ON PERFORMANCE, BLOOD INDICES AND JEJUNAL MORPHOLOGY OF LAYING HENS DURING POST PEAK PRODUCTION

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ABSTRACT: The purpose of this study was to evaluate the effect of dietary supplementation of probiotic (*Lactobacillus acidophilus*), phytobiotics (bay leaves, onion peel and garlic peel) or their combination on blood parameters, morphology of digestive tract and performance of laying hens. The experiment used 144 laying hens aged 72 weeks old, and divided into 6 treatments including Ctl (basal feed as control); Pr (basal feed + 1.2 mL/day of probiotic *L. acidophilus*); Ph2 (basal diet + 2% phytobiotic of diet); PrPh2 (basal diet + 1.2 mL/day probiotic + 2% phytobiotic); PrPh4 (basal diet +1.2 mL/day probiotic + 4% phytobiotic) and PrPh6 (basal diet + 1.2 mL/day probiotic + 6% phytobiotic). Feed intake and egg mass were weekly recorded. One chick from each replicate was blood sampled and then slaughtered for data collection. Results showed that treatments had no effect on hemoglobin, erythrocyte and leukocyte of hens. The control hens had higher levels of cholesterol and LDL than that of PrPh2, PrPh4 and PrPh6 hens. HDL level tended to be higher in PrPh2 and PrPh4 as compared to control hens. Compared to control, the villi height of jejunum was higher in the treated hens, with PrPh4 had the highest villi height. Ileal protein digestibility tended to be higher in the treated than that in control hens. Also, there was a clear tendency that feed conversion ratio was lower in the treated hens than that of control. In conclusion, the combined use of probiotics and phytobiotics improved physiological condition, ileal histomorphology, ileal protein digestibility and feed conversion of laying hens during post peak production.

Keywords: Garlic, Herb, Laying Hen, Phytobiotic, Probiotic.

INTRODUCTION

Egg production declines after laying hens reaching the peak of production. Antibiotic growth promoters (AGP) was often administrated to feed to slowdown the declining rate in egg production after the peak production (Salim et al., 2018). In the digestive system, AGP plays a function in eliminating pathogenic bacterial populations in order to maximize nutrient absorption by hens (Purbarani et al., 2019). In recent years the use of AGP has been banned in many countries, including Indonesia. This is due to several negative effects of AGP, e.g., there is residue that found in livestock products, so that it can endanger human health. These health risks include bacterial resistance, allergies to the products consumed, the high risk of illness from poisoning, etc. (Deko et al., 2018).

Various alternatives to AGP substitute for poultry have been studied, one of which is probiotics (Sugiharto et al., 2017). One of the most widely used probiotic bacteria in the poultry industry *is Lactobacillus achidophilus* (Saputra et al., 2020). Indeed, the use of *L. acidophilus* was reported to improve physiological conditions, health status (Siadati et al., 2017), growth of intestinal villi (Hedayati and Manafi, 2018), nutrient digestibility and performance of poultry (De Cesare et al., 2017). Another alternative ingredient that can be used as a substitute for AGP are herbal ingredient or phytobiotics (Haniarti et al., 2019). Phytobiotic that can be used as feed additive in poultry include bay leaves (Santoso et al., 2017), garlic peel (Benítez et al., 2011) and onion peel (Rahmawati et al., 2019). Previous studies have reported that bay leaf flour added to feed has an effect on the microflora of the digestive tract of broiler chickens (Sjofjan et al., 2019). Meanwhile, the use of garlic and onion peels and their combination in feed could increase the digestibility of feed protein and performance of duck (Saputra et al., 2016) and reduce triglyceride levels in the blood of broiler chicken (Kim et al., 2009). Besides having a high flavonoid content, these herbal ingredients have also been reported to contain oligosaccharides which can act as prebiotics, which are useful for the growth of probiotic bacteria (Babbar et al., 2016).

To increase its effectiveness as an alternative to AGP, probiotics are often combined with other active ingredients such as phytobiotics. Chang et al. (2019) reported that multi strains of probiotics supplemented with herb (*Gardeniae fructus*) can suppress the growth of pathogenic bacteria and increase the growth of the intestinal villi of broiler chickens. The combination of *L. acidophilus* with Dayak onion extract added to feed could also improve nutrient digestibility, health conditions and performance of broilers (Yuanita et al., 2019). Likewise, the combined use of *Lactobacillus* spp. and ginger and turmeric extracts improved production performance and health parameters of broilers (Risdianto et al., 2019).

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To best of our knowledge, the use of a combination of probiotic (*L. acidophilus*) and phytobiotic from bay leaves, onion and garlic peels on laying hens during the post peak production has never been reported. Therefore, the study aimed to determine the influence of dietary administration of probiotic *L. acidophilus*, phytobiotics (bay leaves, onion and garlic peels powders) or their combination on blood profile, jejunal morphology and the performance of laying hens.

MATERIALS AND METHODS

The study used 144 of laying hens (Isa Brown strain) post peak production, aged 72 weeks (weight 1.98 ± 0.1 kg). The hens were raised for 5 weeks (35 days). The feed ingredients used in this study included yellow corn, rice bran, soybean meal, meat bone meal, fish meal, salt, grit, methionine, premix, onion peel (OP), garlic peel (GP) and bay leaf (BL). Feeds were offered to hens *ad libitum* with free access to water throughout the experiment. The phytobiotic combination used was 50% of BL, 25% OP and 25% GP. The probiotic bacteria used was *L. acidophilus* (10^8 cfu/mL). Details of the composition of feed ingredients and treatment are listed in Tables 1 and 2 while the nutritional content of phytobiotics is in Table 3.

This study was arranged according to a completely randomized design with 6 treatments and 4 replications, with 6 hens in each replicate. The treatments included Ctl (basal feed as control); Pr (basal feed + 1.2 mL/day of probiotic *L. acidophilus*); Ph2 (basal diet + 2% phytobiotic of diet); PrPh2 (basal diet + 1.2 mL/day probiotic + 2% phytobiotic); PrPh4 (basal diet +1.2 mL/day probiotic + 4% phytobiotic) and PrPh6 (basal diet + 1.2 mL/day probiotic + 6% phytobiotic).

Weekly feed intake and egg mass were recorded. Feed conversion was calculated by dividing the average feed consumption by the average egg mass for a week. Two mL of blood sample was taken on day 34 of the experiment through the brachial vein and put into a tube containing ethylene diamine tetraacetic acid (EDTA) and stored in a cooling box for further analysis. The determinations of cholesterol, low-density lipoprotein (LDL) and high-density lipoprotein (HDL) levels were conducted based on the enzymatic-colorimetric method. The enumeration of erythrocytes and leukocytes was carried out using a hemocytometer, while hemoglobin levels were determined using the Sahli-Hellige method (Sufiriyanto et al., 2018). At 35 days of study, one hen was taken from each replicate then slaughtered. Shortly after slaughter, the jejunal segment was obtained for the measurement of pH values, length, weight (empty weight) and villi height. Digesta samples from ileum were also taken for the calculation of crude protein content according to the method as described by Lemme et al. (2004).

Table 1 - Ingredients of basal diet

Ingredients	Composition (%)
Maize	54.9
Rice bran	8.84
Soybean meal	19.2
Meat bone meal	6.29
Fish meal	1.00
Salt	0.28
Limestone	7.88
Methionine	0.05
Premix	1.50
TOTAL	100

Table 2 – Nutritional contents of basal diet

Nutrient composition	Contents8
Metabolizable energy (kcal/kg)	2,654
Crude protein (%)	17.3
Crude fat (%)	3.51
Calcium (%)	5.55
Phosphorus (%)	0.66

Table 3 - Nutritional content of phytobiotics (combined phytobiotics)

Nutrient	Compositions (%)
Dry matter	87.55
Crude protein	8.58
Crude fat	1.63
Crude fiber	34.08
Calcium	1.20
Phosphorus	0.28

Statistical Analysis

The data were analyzed using analysis of variance (ANOVA) with a significance level of 5%. When the treatment showed a significant effect ($p<0.05$), the Duncan multiple range test was then performed. The obtained results were expressed as the mean ± standard deviation.

Ethical approval

The *in vivo* study was supervised by the Animal Ethics Committee of the Faculty of Animal and Agricultural Sciences, Universitas Diponegoro and conducted in accordance with the basic animal husbandry and health protocols referred to in Legislation of the Republic of Indonesia No. 18, 2009.

RESULTS

Data on blood profile of laying hens are presented in Table 4. It was shown that treatments had no effect ($p>0.05$) on hemoglobin levels, erythrocyte counts and leukocyte numbers of laying hens during post peak production. Table 5 demonstrates the data on concentrations of cholesterol, LDL and HDL in the plasma of laying hens. The control hens had higher levels of cholesterol ($p=0.05$) and LDL ($p<0.05$) than that of PrPh2, PrPh4 and PrPh6 hens. HDL level tended to be higher ($p=0.08$) in PrPh2 and PrPh4 as compared to control hens. Data on the conditions of jejunal segments of laying hens are presented in Table 6. Compared to control, the villi height of jejunum was higher ($p<0.05$) in the treated hens,

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with PrPh4 had the highest villi height. The treatments had no substantial effect on the relative weight and length as well as pH values of jejunum. Table 7 shows the data on ileal protein digestibility and performance of laying hens. Ileal protein digestibility tended (p=0.06) to be higher in the treated than that in control hens. Also, there was a clear tendency (p=0.05) that feed conversion ratio was lower in the treated hens than that of control. Yet, there was no significant effect of the treatments on the feed intake per week and egg mass per week.

Table 4 - Blood profile of laying hens

Variables	Ctl	Pr	Ph2	PrPh2	PrPh4	PrPh6	p-value
Hemoglobin (g/dL)	7.20±0.12	7.57±0.28	7.20±0.01	7.60±0.07	7.50±0.12	7.40±0.14	0.27
Erythrocytes (x10^6)	2.23±0.12	2.57±0.77	2.75±0.19	2.61±0.24	2.56±0.06	2.64±0.16	0.32
Leukocytes (10^3/mm^3)	9.05±1.11	9.13±1.26	8.98±0.98	8.82±0.60	9.20±0.98	9.07±1.14	0.99

Ctl: basal feed as control, Pr: basal feed + 1.2 mL/day of probiotic *L. acidophilus*, Ph2: basal diet + 2% phytobiotic of diet, PrPh2: basal diet + 1.2 mL/day probiotic + 2% phytobiotic, PrPh4: basal diet +1.2 mL/day probiotic + 4% phytobiotic, PrPh6: basal diet + 1.2 mL/day probiotic + 6% phytobiotic)

Table 5 - Plasma profile of laying hens

Variables	Ctl	Pr	Ph2	PrPh2	PrPh4	PrPh6	p-value
Cholesterol	211±9.1	178±9.1	174±6.9	170±5.2	170±10.5	172±23.3	0.05
HDL	36.26±1.9	44.4±3.6	41.4±2.8	49.6±3.4	45.5±2.9	44.4±2.4	0.08
LDL	112±6.4^a	85.7±14.4^{ab}	83.9±1.9^{ab}	75.3±9.5^b	74.1±13.1^b	61.0±7.7^b	0.04

^{a,b}: Means within a row with different superscripts differ significantly (p<0.05); Ctl: basal feed as control, Pr: basal feed + 1.2 mL/day of probiotic *L. acidophilus*, Ph2: basal diet + 2% phytobiotic of diet, PrPh2: basal diet + 1.2 mL/day probiotic + 2% phytobiotic, PrPh4: basal diet +1.2 mL/day probiotic + 4% phytobiotic, PrPh6: basal diet + 1.2 mL/day probiotic + 6% phytobiotic), HDL: high-density lipoprotein, LDL: low-density lipoprotein

Table 6 - Jejunal segment of laying hens

Variables	Ctl	Pr	Ph2	PrPh2	PrPh4	PrPh6	p-value
Relative weight (% BW)	1.00±0.07	0.93±0.09	0.93±0.06	0.94±0.06	1.00±0.09	1.01±0.19	0.89
Relative length (cm/kg BW)	31.6±0.89	35.0±3.28	32.3±1.23	30.6±0.55	32.6±0.71	33.2±1.76	0.79
pH	6.30±0.09	6.15±0.13	6.08±0.05	6.08±0.02	6.05±0.05	6.05±0.03	0.17
Villi height (µm)	812±88.2^c	971±14.1^b	941±11.1^b	972±13.2^b	1097±20.3^a	1055±10.6^{ab}	<0.01

^{a,b,c}: Means within a row with different superscripts differ significantly (p<0.05), Ctl: basal feed as control, Pr: basal feed + 1.2 mL/day of probiotic *L. acidophilus*, Ph2: basal diet + 2% phytobiotic of diet, PrPh2: basal diet + 1.2 mL/day probiotic + 2% phytobiotic, PrPh4: basal diet +1.2 mL/day probiotic + 4% phytobiotic, PrPh6: basal diet + 1.2 mL/day probiotic + 6% phytobiotic), BW: body weight

Table 7 - Ileal protein digestibility and performance of laying hens

Variables	Ctl	Pr	Ph2	PrPh2	PrPh4	PrPh6	p-value
Ileal protein digestibility	60.5±0.72	70.6±2.43	72.5±8.17	75.5±3.04	75.6±0.01	76.3±0.33	0.06
Feed intake/week	711±10.9	680±9.49	675±11.12	694±34.1	693±31.9	679±7.68	0.83
Egg mass/week	319±16.1	339±4.09	328±7.59	346±22.7	353±22.8	354±12.1	0.59
Feed conversion ratio/week	2.24±0.12	2.00±0.05	2.06±0.03	1.99±0.04	1.97±0.4	1.93±0.09	0.05

^{a,b}: Means within a row with different superscripts differ significantly (p<0.05), Ctl: basal feed as control, Pr: basal feed + 1.2 mL/day of probiotic *L. acidophilus*, Ph2: basal diet + 2% phytobiotic of diet, PrPh2: basal diet + 1.2 mL/day probiotic + 2% phytobiotic, PrPh4: basal diet +1.2 mL/day probiotic + 4% phytobiotic, PrPh6: basal diet + 1.2 mL/day probiotic + 6% phytobiotic)

DISCUSSION

Data on blood profiles of laying hens in this study were consistent with Abudabos et al. (2016) who reported that the use of probiotics, phytobiotics or a combination of both did not have any negative impact on the physiological conditions of laying hens. With regard to probiotic effect, Siadati et al. (2017) used probiotic *Lactobacillus* (*L. crispatus, L. salivarius, L. crispatus* and *L. oris*) and noticed no significant effect on haemoglobin and erythrocyte levels of Japanese quails. Also, Mateova et al. (2008) did not find any effect of *L. fermentum* on the number of leukocytes of broiler chicken. In term of phytobiotics, the addition of phytobiotics (onions, ginger, turmeric and cutcherry) did not significantly affect the levels of hemoglobin, erythrocytes and leukocytes of Tegal ducks and Muscovy ducks in the study of Ismoyowati et al. (2019). In contrast to our present results, Deraz (2018) reported that probiotics (*Lactococcus lactis* ssp. *Lactis* and *Lactobacillus plantarum*) increased the levels of haemoglobin, erythrocytes and leukocytes in broiler chickens. Likewise, the combination of phytobiotics increased the levels of hemoglobin, erythrocytes and leukocytes in laying hens (Tang et al., 2017). The variations in probiotic bacteria species or strains, levels of probiotics, types and levels of phytobiotics, animals used in the experiment and experimental conditions may be attributed to the divergent results above.

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Data in the present study showed that cholesterol and LDL concentrations were lower in the plasma of laying hens treated with the combination of probiotic *L. acidophilus* and phytobiotic based on bay leaves, onion and garlic peels. Indeed, the effect of single administration of probiotic or phytobiotic alone did not affect the levels of cholesterol and LDL in the plasma of laying hens. In this respect, the synergistic or complementary effect between probiotic bacteria and phytobiotic seemed to reduce the levels of plasma cholesterol and LDL. This inference was supported by Rahman et al. (2019) showing the synergistic work of probiotic *Bacillus* and commercial phytobiotic (Galibiotic®) in lowering the concentration of cholesterol and LDL in the plasma of broilers. It has been known that probiotic *L. acidophilus* could produce bile salt hydrolase, which can deconjugate bile salts to lower cholesterol (Jahanian and Ashnagar, 2015). Likewise, flavonoids content in phytobiotics could inhibit *de novo* cholesterol formation. In the latter case, flavonoids can inhibit the formation of acetyl co-A, which is the main precursor for cholesterol formation (Zeka et al., 2017). In this study, HDL level tended to increase with dietary inclusion of probiotic, phytobiotics or combination of both as compared to control. This result was in agreement with Krauze et al. (2020) reporting the effective impact of probiotic *Bacillus subtilis* and phytobiotic (cinnamon oil) in enhancing the HDL level in the serum of broiler chicken. It was most likely that the complementary effect between probiotic and phytobiotic occurred in this study as the plasma HDL level was higher in PrPh2 when compared with that in Pr, Ph2 and Ctl hens.

The laying hens used in this study were quite old (72 weeks of age), and that the growth and development of the intestine of these hens had been stopped. For this reason, the relative length and weight of jejunum were not different across the treatment groups. In general, pH values can be used to indicate the condition of digestive tract, which is related to the numbers of pathogenic bacteria and good bacteria. In this study, the jejunal pH values of hens were within the normal values, as Purbarani et al. (2019) noticed that the pH values of jejunum ranges from 5.80 to 6.90. In this study, the jejunal villi height increased with the dietary administration of probiotics, phytobiotics and their combination. This seemed due to the antibacterial activity of probiotics and phytobiotics, which may reduce the pathogenic bacteria load in the intestinal villi (Hussein et al., 2020). The latter condition may consequently improve the growth and development of the intestinal villi (Hedayati and Manafi, 2017).

In this study, the increased ileal protein digestibility seemed to be attributed to the increased jejunal villi height of hens. The higher the intestinal villi implied in the wider surface area for the nutrient absorption (Fesseha, 2019). This may therefore increase the capacity of the intestine in absorbing the nutrients from feed (Purbarani et al., 2019). Overall, the increase in nutrient digestibility resulted in the improvement of nutrient utilization and hence improved the FCR of layer hens. Yet, the latter improvement did not accompanied by the increase in egg mass produced by the hens. This was consistent with previous study showing that probiotic *Bacillus subtilis* and *Bacillus licheniformis* (Mahdavi et al., 2005) and phytobiotics based on black cumin, thyme, cinnamon, ginger and pomegranate (Soliman and Kamel, 2020) did not significantly affect egg mass as well as feed consumption of laying hens.

CONCLUSION

The combined use of probiotics and phytobiotics improved physiological condition, ileal histomorphology, ileal protein digestibility and FCR of laying hens during post peak production. The combination of 1.2 mL/day of probiotic *L. Acidophilus* and 4% of phytobiotics resulted in the best outcomes for laying hens during post peak production.

DECLARATIONS

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Authors' Contribution
All authors contributed in research and writing, equally.

Conflict of interests
The authors declare that they have no competing interests.

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EFFORTS, SUCCESSES AND CHALLENGES OF GREEN FEED PRODUCTION IN ETHIOPIA

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REVIEW

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ABSTRACT: Improving the feed supply and digestibility of roughage through green feed supplementation was the aim of forage production in Ethiopia. In spite of the time-consumed efforts made, the success is still low. This review summarizes efforts, challenges, good practices and indicates where the focus of future efforts should be. The efforts made in improve forage production (IFP) includes selection of forage species and development strategies, preparation of extension manual, training of stakeholders, formulation of forage seed production system, and introduction of hydroponic fodder. As a result farmers increased milk production and reduce feed cost by 20% and 40 %, respectively, and fatten ruminants in 2 to 3 cycles per year as a result of improved forage use. However, there is no management model for communal grazing land. IFP constrained by long dry period, scarcity of land, irrigation and the subsistence system of livestock production and the free grazing practice. Consequently, the communal grazing lands are devastatingly overstocked and degraded, livestock become dependent on crop residues, IFP practice remains low and livestock feed deficit is common. Therefore, it is recommended that future efforts should focus on designing communal grazing land tenure model and development package. Green forge production (GFP) should concentrate on livestock excluded areas and tree legumes to produce green feed in the dry period. GFP best practices should be expanded to the areas similar to that the practices found effective. Reduce feed deficit by improving crop production to increase the crop residue and the grain yield. The subsistence system of production should be transformed into market-oriented by introducing a technology package that reduces young stock mortality, increases fertility, calf/ lamb crop and livestock offtake rate. Technical simplicity and cost-effectiveness, of the hydroponic fodder system, should be assessed before implemented in a wider setting.

Keywords: Animal Nutrition, Forage, Grazing, Green feed, Livestock.

INTRODUCTION

Improved forage production started in Ethiopia in 1950s (Fikre, 2018). In spite of its long history and existence of some exemplary practices, the contribution of improved forage to the total feed resource is still low (0.32 %; CSA, 2018). The communal grazing lands are ownerless and devastatingly overstocked and degraded due to the absence of a clear model that indicates the right and obligation of users. Livestock become increasingly dependent on crop residues (Stark et al., 2018; Atsbha et al., 2020). Feed supply deficit is common in the mixed crop-livestock production system of Ethiopia.

The objective of improved forage production in Ethiopia was to increase feed supply and improve the intake and digestibility of the poor quality hay and the crop residues through green feed supplementation. The net digestibility of dry forages can be increased when fed together with 25% of fresh green material (Horrocks and Vallentine, 1994). The total digestibility of dry matter increased when 10 to 30% of the concentrated portion of the ration replaced by green feed in the form of hydroponic fodder (AL-Saadi et al., 2015).

Young, dark green forage tends to contain more minerals than old, dry, yellowing forage (Lukuyu et al., 2007). They also contain vitamins or raw material for vitamin synthesis in the rumen. Green fodders are good sources of α-tocopherol (the most biologically active form of vitamin E), young grass being a better source than mature herbage. The leaves contain 20–30 times as much vitamin E as the stems (McDonald et al., 2007). Losses during haymaking can be as high as 90 percent, but losses during ensilage or artificial drying are low (McDonald et al., 2007). Silage and hay contain 20 to 80 percent less α-tocopherol than does fresh forage (NRC, 2001).

Vitamin E involves in the maintenance of cellular membranes, immunity, and reproductive function. White muscle disease is a classic sign of a clinical deficiency of vitamin E (NRC, 2001). Vitamin E deficiency also found to have a relationship with mastitis, and immune function (NRC, 2001). Green herbage is also an exceptionally rich source of β-carotene, a precursor of vitamin A, and the dry matter of the young green crop may contain as much as 550 mg/kg (McDonald et al., 2007). Vitamin A is necessary for low light vision, normal growth and development (including fetal growth), spermatogenesis, and for maintenance of skeletal tissue and epithelial tissue (NRC, 2001). Abortions, increased prevalence of retained fetal membranes, and increased calf morbidity and mortality are indicators of vitamin A deficiency in gestating cows (NRC, 2001).

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Green feeds also provide vitamin K to animals (McDonald et al., 2007). Cattle require vitamin K for the synthesis of at least a dozen proteins; among these are four blood clotting factors; prothrombin (factor II), and factors VII, IX, and X (NRC, 2001). These vitamin K dependent protein factors are components of a complex system that functions to prevent hemorrhage by activation of thrombin and ultimately clot formation (NRC, 2001). Green feed reduces Methane emission to the environment (Prusty et al. 2014; Mohini et al., 2007). The enteric methane emission was reduced by 5-12% on feeding green fodder based ration to river buffaloes (Prusty et al. 2014). Mohini et al. (2007), reported lower CH4 emission (218.15 vs. 233.50 and 252.82 g/d) in lactating cows fed only fresh green fodder and concentrate mixture as compared to dry roughage fed cows. The inhibition of hydrogen-producing bacteria that are accounted for the metabolization of pyruvate to acetyl CoA might also be responsible for the reduction of methane emission on feeding green fodder (Hossain et al., 2017). Nathani et al., (2015) also reported that increasing green fodder proportion in the diet might have also reduced the active genes for the production of methane by reducing the methanogenic archaea population in the rumen. Dairy animals producing up to 5-7 liters of milk per day can be maintained exclusively by feeding green fodders (Hossain et al., 2017).

To increase green feed supplementation practices, immense efforts have been made in selecting and introduction of different forage species in different agro-ecological zones of Ethiopia (Alemayehu, 2006). However, apart from some exemplary achievements observed, the practical use of improved forage crops for animals is still low in many areas (Mekoya, 2008). The objective of this review is to summarize the efforts, challenges, and available good practices in green feed or forage production and indicate where the future efforts should focus.

DISCUSSION

Efforts of green forage production in Ethiopia

Forage development for livestock has a long history in Ethiopia going back to the 1950s at Jimma and moving on through activities at Haramaya University (Fikre, 2018). The forage and pasture seed production began in 1970 in Ethiopia by Arsi Rural Development Unit (ARDU/ CADU) (Alemu, 2011; Mengistu and Assefa, 2012). Oats, vetch, Rhodes grass, Phalaris, Panicum, Buffelgrass, Elephant grass, Green leaf desmodium and Fodder beet were produced in Kulumsa, Dera, Bekoji, Assela and other livestock farms (Fikre, 2018).

The contractual system of forage seed production was initiated by the Fourth Livestock Development Project (FLDP) in 1987/88 on the objective of producing better quality seed at lower prices and greater quantities in centrally controlled seed production systems using individual farmers and farmers cooperatives (Fikre, 2018). The program placed emphasis on the production of forage legume seed, with a capacity of 100-120 tons of forage seed per year during its period of operation (Grass and Turner, 1997, Alemayehu et al., 1994). The contractual forage seed development system functioned well during the project lifespan, but after the project phased out, seed production-related activities failed to operate as successfully as they did during FLDP (Fikre, 2018). The forage seed production intervention was continued by Smallholder Dairy Development Program (SDDP) from 1987-1991 in Heifer distribution together with forage/seed (Yimer and Kidane, 2015).

Various improved forage production strategies were developed and implemented. The strategies evolved from experiences of other countries and an understanding of the importance of matching forage systems to different agro-ecological zones of Ethiopia (Alemayehu et al., 2017). Almost all the strategies emphasize the cut and carry system of feeding, on the objective of providing succulent green forage thereby maintaining the health of soil conservation structures on which the forage planted.

The strategies have presented in the forage extension manual of FLDP. In addition to the strategies, the manual contains the basic guidelines for forage development and management at the farm level. The manual is written for Development Agents in the ministry of agriculture but is useful for all extension workers (FLDP, 1989). The commercial green feed has been produced by YB Plant Micropropagation PLC owned by Behaylu Abraha in the low-cost hydroponic fodder production system in 2014 in Ethiopia. The number of dairy farm owners relying on Behaylu for hydroponically produced barley fodder has grown steadily, reaching 400 milking cows (LIVES, 2014). Hydroponics fodder is a succulent fodder without soil but in water or nutrient-rich solution in a greenhouse (hi-tech or low-cost devices) for a short duration, 7 to 10 days (Naik et al., 2015).

Since a couple of decades ago, hydroponic fodder production technology has been advocated as a solution for conventional green fodder production challenges and as a climate change adaptation strategy (Muthuramalingam et al., 2015) in many countries. On the contrary, other groups of authors argue that the profitable use of sprouting grain as a feed source for commercial cattle production appears unlikely (Sneath and McIntosh, 2003) or totally have no advantage (Fazaeli et al., 2012). The major reason for tending to discourage hydroponic fodder production by these groups includes reduction in dry matter (DM) weight and greater cost as a result of sprouting. Nevertheless, some other authors noted that it is the ultimate animal performance relative to the alternative costs that determine the profitability and usefulness of hydroponic fodder (Tranel, 2013), which indicates that the usefulness of hydroponic fodder depends on local condition. Although such dialogue exists among professionals, hydroponic fodder production technology was introduced to some parts of Ethiopia without any preliminary study.

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Successes of forage production in Ethiopia

In the area, where market for forage or animal products available improved forage production practice is suitable. A study made by Mesfin et al. (2013a,b) in such area namely Girar Jarso, Tiyo, chiro district farmer allocate 400 m^2 to one hectare per household for forage production. Farmers reported that the improved forage used in feeding dairy and fattening animals increased their income, reduced their feed cost due to the decrease in concentrate cost and ensured the year-round feed availability. Good animal management practice reduced the disease problem during animal breeding. According to the interviewed farmer, milk production may increase by about 20 % while feed cost reduced by about 40 % (Mesfin et al., 2013a). In the irrigated sites, farmers have been able to harvest elephant grass forage year-round, usually at monthly or bi-monthly intervals. Due to the year-round fodder availability, farmers are able to fatten cattle and/or small ruminants 2 to 3 cycles per year and milk yield of local cows increased from 1 to 3 liters/cow/day (Mesfin et al., 2013b). Moreover, elephant grass planted along the edges of irrigation canals have also improved the feed availability particularly during the dry period and help in stabilizing the irrigation canal thereby reducing water loss through evaporation (Mesfin et al., 2013b). Additional income obtained from the sale of planting materials of elephant grass was another benefit, even though such benefit is rarely obtained but its future potential market looks promising (Mesfin et al., 2013b).

Challenges of forage production in Ethiopia

Even if exemplary improved forage production practices found in some pocket areas of Ethiopia, the contribution of improved forgeries to the total feed resources is still very small. According to CSA (2018) improved forage production covers only 0.32% of the total feed resources of livestock in rural peasant farmers of Ethiopia. The failures of green feed production in many places are due to scarcity of land and water for irrigation. In the highland of Ethiopia, where mixed crop-livestock production system exists, farmers give priority in allocating land more often to crops than forage production. There is no market-oriented livestock production in most of the mixed crop-livestock production systems. Cattle are primarily kept to supply draft power needed for crop production (FAO, 2018). Small ruminants are maintained as a means of savings and meat for household consumption (Gizaw et al., 2010). Inadequate feed supply is one of the major constraints hampering market-oriented livestock development in Ethiopia (Firew and Getnet, 2010).

In Ethiopia, most farmers practice free grazing except in areas, such as Harerghe, where grazing lands are limited in size and where the farming system favors the growth of perennial cash crops (Gebregziabher and Gebrehiwot, 2011). Damage of improved forages planted by some farmers with the animals of the others due to the open grazing system practice is one of the reasons for the low adoption of forage development in many areas of Ethiopia. Efforts made to introduce zero (controlled) grazing in 2006. The strategy was to adopt what Hararghe farmers have been practicing for years. However, this has been faced by resistance by farmers which has resulted in a low adoption rate. Farmers relate zero-grazing with grazing land confiscation (Gebregziabher and Gebrehiwot, 2011).

The increasing expansion of land cultivation for crop production (Amsalu et al., 2007; Bogale et al., 2008) and construction of public institutions at the expense of grazing land has resulted in shrinkage of the area. Grazing lands are devastatingly overstocked and that the herbage biomass productivity is declining both in quantity and quality (Hassen et al., 2010; Tschopp et al., 2010; Alemayehu, 2013). In general, crop residues are increasingly becoming the main sources of livestock feed resources in the mixed crop-livestock production systems (Bogale et al., 2008; Duncan et al., 2016).

Overall, about 51, 19 and 38% annual feed deficit in DM, ME and DCP, respectively were reported in the central high land of Ethiopia (Mekete et al., 2018). Feed supply deficits in mixed crop-livestock production system have also reported by Kassa et al. (2003) and Wondatir et al. (2011), in Gummara-Rib watershed of the Lake Tana in Amhara region, and Harerge highland, respectively. In most parts of Ethiopia, the livestock management practice is a low-input, low-output traditional extensive system. During the FLDP life, the price of forage seed was not governed by the free market. The project purchase with higher price from seed producer farmers and supply to the forage producers free of charge or with a highly subsidized price. When the project phase-out efforts of promoting forage seed production and dissemination did not sustain as the agricultural offices did not have sufficient grant money to purchase and supply seed. Forage seed production also ceased because farmers chose to use their land to produce other types of seed and crops that had a more significant impact on their livelihoods (Tekalign, 2014). This faded the glimmered hop of green feed production in conventional feed production in Ethiopia.

CONCLUSION AND RECOMMENDATION

It is concluded that the absence of a clear model for use right and development obligation makes the communal grazing land ownerless and devastatingly overstocked and degraded. Even there are some exemplary practices, the long dry period, absence of irrigation and land scarcity coupled with low input low output subsistence system of livestock production put the improved forage production difficult in rural Ethiopia. Therefore it is recommended that future efforts should focus on selecting, testing and implementing a communal tenure model that is suitable to communal grazing lands in Ethiopia and improve the productivity of grazing lands by over-sowing, differed grazing and controlling stocking rate as per the caring capacity of the grazing lands. Improved green feed/forage production should concentrate on livestock excluded areas. Emphasis should be given to tree legumes to produce green feed in the dry period to be used by a cut and carry system.

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Green feed production best practices should be expanded to the areas similar to that the practices found effective. Organize farmers' visit and open discussion among farmers. Reduce feed deficit by increasing livestock off-take during a time of scarcity and multidimensional crop improvement to increase the quantity and feeding value of crop residue without negatively affecting the grain yield. Finally, gradually transform the low-input low output livestock production to the market-oriented system by formulating and introducing a multidisciplinary livestock technology package that reduces young stock mortality, increases fertility that increases the calf/ lamb or kin crop to be sold and finished in urban and peri-urban areas where better agro-industrial byproducts available. The compatibility of hydroponic fodder to the local conditions including its technical simplicity, cost-effectiveness, and performance of locally available seeds in the hydroponic system should be assessed before expanding the technology throughout the country.

DECLARATIONS

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Authors' contribution

The author contributed alone to this work starting from reviewing publishes articles to preparation of the manuscript.

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Conflict of interests

The author declare that there is no conflict of interests on this work

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EFFECT OF LACTATION LENGTH AND LITTER SIZE ON MILK COMPOSITION OF BOER GOAT WITHIN ONE MONTH OF PARTURITION

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RESEARCH ARTICLE

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ABSTRACT: The aim of this study was to determine the effect of lactation length and litter size on the milk composition of Boer goats. Milk samples from 23 lactating Boer goats reared in NLBO (National Livestock Breeding Office), Pokhara, Nepal, were collected and analyzed in a laboratory present within a farm. Goats were reared under the same environment and provided with similar care and management. Lactation length showed a non-significant effect on fat content in the milk whereas all other constituents were influenced significantly. Similarly, litter size showed a significant effect on the freezing point of milk only. Litter size and all milk constituents were negatively correlated. However, Litter size and freezing point showed moderately strong correlation. Twinning did not significant influence fat, protein, lactose and solids nonfat content of goat milk. All constituents were weakly correlated to litter size however freezing point showed moderately strong correlation. Thus, more focus must be given to the nutritional management of triplets and twins to improve their growth rate compared to a single kid.

Keywords: Boer goat, Lactation length, Milk composition, Litter size, Twinning.

INTRODUCTION

Boer goats (*Capra aegagrus hircus*) are one of the most desirable goat breeds with good meat conformation, short hair and a variety of color patterns and are believed to be the result of a genetic pooling of African indigenous goats, Indians goats, Angora goats, and European dairy goats (Lu, 2001). Boer goats were believed to be brought in Nepal around 1999 A.D. Boer goats started being reared for detailed research in Goat Research Center, Bandipur in 2008 A.D (Nepali, 2016). Its demand has been increasing worldwide for its excellent body conformation, fast-growing rate, and good carcass quality (Lu, 2001). Boer breed has higher immunity to diseases and quicker maturity than our local breeds. The growth rate of Boer kids was found to be 150- 300 grams per day (Nepali, 2016). The average growth rates were also recorded as 291, 272, 245, and 250 g/day in male goats and 272, 240, 204, and 186 g/day in female goats from birth to 100, 150, 210, and 270 days of age respectively (Lu, 2001). Multiple kids can easily be reared as it produces an adequate amount of milk during lactation.

Two percent of the world's total annual milk supply is maintained by goats. Goat milk possesses higher digestibility of protein and fat, alkalinity, buffering capacity, and certain therapeutic values over a cow or human milk (Park, 2009). Goat milk has significantly higher protein and ash, but lower lactose, than human milk (Park, 2009). It can therefore be considered as the main replacement of cow's milk for lactose intolerant individual (Kalyankar et. al., 2016). Goat milk is richer in caproic, caprylic, and capric acids than cow milk with 6, 8, and 10 carbon atoms, respectively (Chilliard, 1997). It is composed of medium-chain fatty acids (8-12 carbon atoms) that are efficient antimicrobials (Desbois and Smith, 2010). Goat milk is rich in calcium and phosphorus and is consumed in many regions, such as the Middle East, southern Asia, and some tropical countries.

Milk composition is affected by multiple factors like age, breed (Sung et al., 1999), nutrition, parity, stage of lactation (Ciappesoni et. al., 2004), environment, fodder, the season of kidding (Crepaldi et. al., 1999), etc. The main aim of this paper was to determine the effect of lactation length and litter size on milk constituent's i.e. fat, lactose, Solids Non Fat (S.NF), protein, and freezing point. Various researches have been carried out to determine the composition and factors affecting the composition of goat milk. However, there is limited information specific to Boer goats in the Nepalese environment during the early stage of lactation.

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MATERIALS AND METHODS

Study area

Milk samples were collected from Boer goats reared under the intensive system in NLBO, Pokhara primarily for research purpose and up-grading of local breed. The research site is located within longitude 83° 58' 20.604" E and latitude 28° 15' 48.996" N at an altitude of about 793m above sea level (Figure 1). Twenty three Boer goats were lactating at the time of data collection. These therefore constituted the sample population. All goats were kept under the same environmental conditions and were provided with similar care and nutrition. Goats were fed with 600 gm of concentrate per day. Ipil-Ipil (*Leucaena leucocephala*), *Melia azadirachta, Ficus semicordata,* etc. and Oat (*Avena sativa),* Sudan, Berseem, etc. were the major sources of fodder and forage respectively provided to the goats.

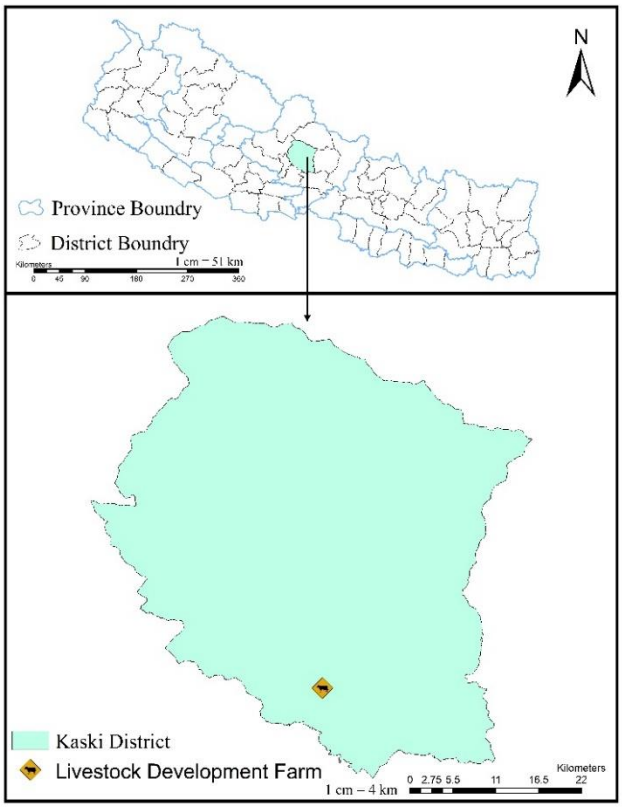

Figure 1 - Map showing livestock development farm in Pokhara, Nepal.

Sample collection procedure

- Goats were handled by two individuals in which one restrains the goat while the other one performs milking operation by hand.
- The teats were washed with clean water and rubbed with a clean towel to remove excess water.
- A total of 10 ml of milk sample was collected from both teats in a sterile container to analyze the milk composition.
- The teats were cleaned again after milking was completed.

The collected milk sample was then taken to the laboratory located within the farm. The samples were homogenized before analysis using Akashganga Milk Analyzer, AMA-Mini-40. The data obtained were recorded and analyzed to determine the effect of lactation length on milk constituents.

Data analysis

Data regarding the age and lactation length was obtained from the recoding system present within the farm. The obtained data were recorded in an Excel sheet and were analyzed using IBM SPSS version 20 (Amos, 2011). The lactation length was divided into three categories as 2nd, 3rd, and 4th week of lactation. The relation between lactation length and milk constituents was analyzed using ANOVA test whereas an Independent sample T-test was used to determine the relationship between litter size and milk composition.

RESULTS

Effect of lactation length on milk composition

The effects of lactation length on milk composition are shown in Table 1. The study revealed that there was no significant effect (p=0.232) of lactation length on the fat content of milk during the first month of lactation. The lowest fat percent was recorded on the 4th week of lactation. Lactation length showed a significant effect (p=0.041) on the Solids nonfat (S.N.F) content. Solids nonfat slightly decrease in 3rd week but was elevated again on 4th week. The freezing point showed a significant difference (p=0.041) due to variation in lactation length. The trend in freezing point indicates a constant decrease with increase in lactation length. A significant effect (p=0.042) of lactation length on the protein content of milk was also recorded during our study. Protein content was reduced during the 2nd, 3rd, and 4th week of lactation respectively. The lactose content of the milk was significantly affected (p=0.049) by lactation length. Lowest lactose content was recorded at 3rd week of lactation based on our study

Effect of litter size on milk composition

Results of our study revealed that fat, S.N.F, protein, and lactose content of milk showed no significant difference within 1 month of parturition due to variation in litter size (Table 2). All components are weakly correlated (negatively) to litter size except freezing point. Goat milk from does with two kids showed slightly lower fat, protein, lactose, and S.N.F content but insignificant compared to a goat with single kids (Table 2). The freezing point of milk showed significant variation (p=0.043) with the number of kids. The freezing point was negatively correlated (r= -0.424) to litter size similar to other milk constituents.

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Table 1 - Table showing the effect of lactation length on milk composition of Boer goats

Composition \ Lactation period (in days)	7-14	15-21	22-30	p-value
Fat (%)	5.963±1.983	6.150±1.801	4.440±1.582	0.232
S.N.F (%)	10.438±0.466	9.930±0.414	10.040±0.182	0.041
Freezing point (Fp) (°C)	0.692±0.048	0.654±0.024	0.648±0.014	0.041
Protein (%)	3.788±0.181	3.610±0.152	3.600±0.071	0.042
Lactose (%)	5.700±0.278	5.420±0.229	5.460±0.114	0.049

Mean ± SD ; Significant at $p < 0.05$

Table 2 - Table showing the effect of litter size on milk composition of Boer goats

Composition \ No of kids	Single	Twins	Correlation coefficient (r)	p-value
Fat (%)	6.44±2.357	5.39±1.587	-0.263	0.225
S.N.F (%)	10.357±0.556	10.031±0.366	-0.343	0.109
Freezing point (Fp) (°C)	0.689±0.056	0.656±0.020	-0.424*	0.043
Protein (%)	3.757±0.336	3.631±0.130	-0.350	0.101
Lactose (%)	5.657±0.336	5.469±0.199	-0.346	0.106

Mean ± SD, Significant at $p < 0.05$, *denotes correlation is significant at 0.05 level.

DISCUSSION

The study performed by Idamokoro et al. (2017) was in line with our findings showing that the milk composition including fat showed no significant difference ($p > 0.05$) during the early, mid, and late stages of lactation. The mean fat percent of Boer milk was calculated to be 4.7% (Mestawet et al., 2012) which was lower than the result obtained within our study. The lower level of fat in goat milk is due to an increase in the molar percentage of propionic acid and the decline in the molar percentage of acetic acids found in the rumen (Morand-Fehr and Sauvant, 1980). But some researches in goats showed that the fat content in the milk gets slowly decreased in 30 days experimental period. The reason behind the decrease of milk fat can be explained by the increase in the percentage of monounsaturated fatty acids with the progress of lactation from 28.2% in colostrum to 33.0% at 30 days post-partum at the expense of polyunsaturated fatty acid which is the main component of milk fat (Marounek et al., 2012). The average fat globule size of goat milk (3.5 µm) is significantly smaller than that of cow milk (4.5 µm) and goat milk possesses a higher percentage of small fat globules than that of cow milk (Knights and Garcia, 1997).

The average SNF content in the milk of Boer goats was observed to be 9.20±0.40% which is lower compared to our findings (Idamokoro et al., 2017). Mean S.N.F content of Boer goat's milk raised under the intensive and extensive system was obtained to be 10.4 ± 6.5% and 10.7 ± 5.1% respectively (Greyling et al., 2004). The research performed by Simos et al. (1996) explained that S.N.F content in the milk was determined by genetic factors rather than the energy intake of an animal.

The correlation coefficient between the freezing point and lactose content in the Boer milk was obtained to be 0.930 (strongly correlated) which is in line with the finding of Janštová et al. (2019) stating that freezing point of goat milk is influenced mainly by lactose and chlorides content in the milk. The proportion of milk constituents in a true solution determines the freezing point of milk and whose proportion of those constituents are affected by multiple factors like breed, the occurrence of subclinical mastitis, nutritional deficiencies, stage of lactation, water intake, thermal stress, and seasonal variation, presence of CO2 in milk, etc. (Slaghuis, 2001). The freezing point of the milk helps to determine the adulteration of water in the milk as it is the least variable component of milk in normal conditions (Zee, 1982).

Milk protein content tends to increase along with the advancement of the lactation period but this outcome didn't hold the truth in case of milk within a month after parturition based on the result obtained during our study (Singh, 1990). Proteins are composed of different amino-acids with 0.7–1.0%N and are influenced by breed, stage of lactation, feeding, climate, parity, season, and udder health status. Milk fat and protein content were determined mainly by the dietary energy balance (Sauvant et al., 1987).

A maximum of 5.27 ± 1.05% lactose was recorded in the intensive feeding of Boer goats in week 5 which is in line with our results (Banda et al., 1992). The highest mean daily milk lactose content (5.0 ± 0.7%) was recorded by Boer does managed intensively (Greyling et al., 2004). Goat milk is often considered as a viable dairy option to those infants showing allergic reactions to both cow milk and soya-based formula because of its lower lactose content for lactose intolerant individuals. Our result was in agreement with Singh and Sengar (1979), who observed that lactose content showed a tendency to decline as the lactation period progresses.

The study of Zamuner et al. (2020) explained that the goats with single kids produce milk with higher fat% and protein% than goats delivering multiple kids. The subsequent decrease in milk fat, protein, lactose, and S.N.F content of Boer goat with single, twins, and triplets was recorded respectively (Lacasse et al., 2014). Goats nursing two kids produce

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more milk (Non-significant) than a single kid (Alkass and Merkhan, 2011). Milk yield and milk composition are negatively correlated (Simos et al., 1991). Suckling reflex post-partum and the physiological mechanism during pregnancy prepares udder to produce more milk for does carrying multiple fetuses (Macciotta et al., 2008). Some contrasting theories are also present explaining that there is no effect of litter size on milk composition (Carnicella et al., 2008). Several studies had suggested that seasonal variations such as temperature, relative humidity, rainfall, and solar radiation/photoperiod showed a significant effect on milk physicochemical composition (Clark and García, 2017). Mammary development and subsequent milk production and its composition are influenced by pre-partum photoperiod in dairy cows, ewes, and does.

CONCLUSION

Lactation length showed a significant effect on lactose, solids nonfat, protein, and freezing point of milk except for fat content in Boer goats within 1 month of parturition. Besides, litter size had a significant influence on the freezing point of milk. Twinning did not significant influence fat, protein, lactose and solids nonfat content of goat milk. All constituents were weakly correlated to litter size however freezing point showed moderately strong correlation. Thus, more focus must be given to the nutritional management of triplets and twins to improve their growth rate compared to a single kid.

DECLARATIONS

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Competing interest

We have no conflicts of interest to disclose concerning the research, authorship, and publication of this article.

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Authors' contribution

S.Regmi performed conceptualization, methodology, analysis and writing-original draft; P.Mahato performed conceptualization, methodology, analysis; Kh.R. Sapkota performed writing- review editing.

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CHARACTERISTICS OF OSTRICH MEAT IN MANUFACTURING SAUSAGE IN COMPARISON WITH BEEF

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ABSTRACT: Ostrich meat has become one of the most popular meat around the world. The objectives of this study are to evaluate the quality characteristics of processed meat, compared to products made from beef. The Ostrich and beef meat was prepared and stored frozen. Sausage was processed in laboratory and analyzed for chemical composition (crude protein, moisture, fat and ash). Physical properties include Water-holding capacity (WHC), cooking loss and microbiological analysis were done. The findings of this study revealed that, chemical composition of sausage from ostrich and beef had highly significant differences in moisture, crude protein, lipids and ash among all types of products. Physical attribute of sausage revealed that, there were highly significant differences among all types of products. The microbiological in sausage observed that, the highly significant differences in *E. coli*, *staphylococcus aurous*, total coli form, yeast and mold and total viable count but was not significant differences in Pseudomonas among all types of sausage. The study recommended that ostrich meat can be used on manufacturing of meat as an alternative to beef meat in limited amount.

Keywords: Animal Product, Meat, Ostrich, Processing, Sausage.

INTRODUCTION

Today, the world faces the problem of shortage in food supply, which creates the malnutrition problem and its consequences in the developing countries, processed meat from non- conventional meat sources like wild animals spatially Ostrich can used as part of solving the problem (Food and Agriculture Organization, 2011). Sausage is prepared food usually made from ground meat, animal fat, salt, spices and typically backed in a casing (Steven et al., 2019). Commonly, there are six types of sausages: fresh sausage, uncooked smoked sausage, cooked smoke sausage, and cooked sausage, dry and semi-dry sausage (Hasan Hussein et al., 2019; Steven et al., 2019).

According to Hoffman et al. (2005) meat from ostriches fed a standard diet contains 21.65, 1.95 and 1.2% protein, fat and ash, respectively. Ostrich meat is recommended for overweight people and for those who suffer from coronary heart disease (Cooper 1999; Antunes et al., 2018; Zdanowska-Sąsiadek et al., 2018). Thus, during cooking, the fat content increase and causes decrease in moisture (Sales et al., 1999). Compared with other species, e.g. chicken and beef, ostrich meat shows a beneficial fatty acids profile (Sales and Horbanczuk, 1998).

Atef et al. (2015) were found that, the chemical composition of beef sausage was: moisture (65.31%) crud fat 20%, ash 9.5% and crud protein 61.28% when investigated effect of Arak Stems Extracts on chemical characteristics bacteria activity evaluation of beef sausage products. Alamin (2015) studied sausage some quality attribute, she found that water holding capacity of beef sausage was 1.06% and the cooking loss was (22.02%). Ibrahim, (2008) reported that Beef Sausage water holding capacity was 59.55% and cooking loss was 17%. Atef et al. (2015) found that the microbial count of beef sausage was: E. coli: N.D, Salmonella: N.D, *staphylococcus aureus*: N.D, Coli form group D and total viable count was (6.15%). The objectives of this study are to evaluate the quality characteristics of processed meat, compared to products made from beef and meat with other meat animals.

MATERIALS AND METHODS

The ostrich and beef meat was obtained and then stored and frozen. The additional materials needed in the formulation were spices, salt, potatoes, onion, garlic, Chick Peas, milk Powder, Bread crumb and Rice.

Two types of sausage (Ostrich and beef) were produced. The minced meat (ostrich, beef) was mixed with other ingredients (Beef fat, chick peas, rice, water, salt, skim Milk powder and seasoning mixture). The mixture was stuffed into prepared sheep intestine casing and formed into fingers of about 5–7 cm in length. Samples of the two types of sausage were analyzed to evaluate the chemical composition, Physical composition, microbial load and sensory evaluation.

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Proximate chemical composition for determination of moisture, crude protein, fat (ether extract) and ash of the sample was done according to A.O.A.C. (2002). The cooking loss was determined according to Honkel (1998) method. WHC determination was done according to the method of Babiker and Lawrie (1983). Microbial load on (sausage) was assessed, it includes the identification of total viable counts, coliforms, yeast and mould, pseudomonas, *E. coli*, *Salmonella ssp*, *Staphylococcus aureus*.

Statistical analysis

All statistical analysis was done by using the computer with SPSS program Ver. (17, 2008) and Duncan multiple range tests was used to detect difference between means (Snedecor and Cochran, 1980).

RESULTS AND DISCUSSION

Table 1 showed the chemical composition of sausage from ostrich and beef. The moisture of sausages processed from ostrich and beef were 66.99% and 68.95% respectively. There were highly significant differences in moisture (P≤ 0.01) among all type of sausages. The higher moisture was found in beef sausage, while the lower moisture was recorded in ostrich sausage. In this study the moisture content was greater than that of Atef et al. (2015), who reported that moisture of beef sausage was (65.31%) and less than Mohammed (2009) who observed that moisture of beef sausage was (72.43%) and disagree with Ibrahim (2008) who recorded that moisture of beef sausage was (57.56%).

The protein of sausages processed from ostrich and beef were 19.25% and 14.00% respectively. There were highly significant differences (P≤0.01) in protein among the products. The higher protein content was observed in ostrich sausage, while the lower protein was found in beef sausage. The findings were lesser than Atef et al, (2015) who were pointed out that the crude protein (CP) of beef sausage was (61.28%) and agreement with Ibrahim (2008) who found that CP of beef sausage (14.09%), but disagree with Mohammed (2009) who stated out that CP of beef sausage was (17.55%).

The ether extract of fat of sausage processed from ostrich and beef were 1.83%, 0.83% respectively. There were highly significant differences (P ≤ 0.01) in total energy expenditure (E.E) of fat among all type of the product. The higher E.E of fat content was observed in ostrich sausage. The findings were very lower than Atef et al. (2015) who found that Fat of beef sausage was 20%, also lesser than Mohammed (2009) who pointed out that Fat of beef sausage was (7.79%) And disagree with Ibrahim (2008) who reported that Fat of beef sausage was (16.49%).

Ash content of sausage processed from ostrich and beef were 2.44% and 2.04% respectively. There were highly significant differences (P≤0.01) in ash between ostrich sausage and beef sausage. The higher ash was found in ostrich sausage. This finding was lower than that of Atef et al. (2015) who found that Ash of beef sausage was (9.5%), but agreement with Mohammed (2009) who stated out that Ash beef sausage was (2.02%) and greater that Ibrahim (2008) who observed that Ash beef sausage was (1.07%).

Table 1 - Average values (Means ± SE) of chemical composition for sausage of ostrich and beef.

Variables	Over All	Sausage		Sig.
		Ostrich	Beef	
Moisture %	68.47±0.29	66.99±0.41^{b}	68.95±0.41^{a}	**
Crude Protein %	16.63±0.004	19.25±0.006^{a}	14.00±0.006^{b}	**
Ether. Extract %	1.33±0.004	1.83±0.006^{a}	0.83±0.006^{b}	**
Ash %	2.24±0.01	2.44±0.02^{a}	2.04±0.02^{b}	**

* The differences between mean of quantitative parameters are marked by various letters in the same raw are significant (P≤ 0.05). ** Mean there is a highly significant differences (P≤ 0.01).

Table 2 showed some physical properties of sausage from ostrich and beef. The water holding capacity of sausage processed from ostrich and beef were 2.24, 3.35 respectively. There were highly significant differences (P≤0.01) in water holding capacity content among sausage. The higher water holding capacity content was observed in beef sausage. The findings were greater than Alamin (2015) who found that the water holding capacity of beef sausage was (1.06%) when was studied sausage some quality attribute, but lower than Ibrahim (2008) who stated out that the water holding capacity of beef sausage was (59.55%).

Cooking loss of sausage processed from ostrich and beef were 32.41, 39.95 respectively. There were highly significant differences (P≤0.05) in cooking loss between ostrich and beef sausage. The higher cooking loss was found to be in beef sausage. The findings were very higher than Alamin (2015) whom reported that cooking loss of beef sausage was (22.02%) when investigated some quality attribute, also greater than Ibrahim (2008) who found that cooking loss of beef sausage was (17%).

Table 3 showed the microbiological properties for sausage of ostrich and beef *E. coli* of sausage processed from ostrich and beef were zero (MPN/g), 5 (MPN/g) respectively. There were highly significant difference (P≤0.01) observed

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among ostrich and beef sausage. The higher *E. coli* content was found to be in beef sausage. The finding was in line with Hamad (2010) who found that *E. coli* count of beef sausage was (20%) when studied prevalence of *Escherichia. Coli*, salmonella and *Staphylococcus aureus* and processed meat in Khartoum State, but disagree with Atef et al. (2015), who was not detected *E. coli* bacteria.

Table 2 - Average values of some physical properties for sausage of Ostrich and beef (Means ± SE).

Variables	Over All	Sausage		Sig.
		Ostrich	Beef	
Water holding capacity	2.80±0.004	2.24±0.006^{b}	3.35±0.006^{a}	**
Cooking Loss	36.18±0.004	32.41±0.006^{b}	39.95±0.006^{a}	**

* The differences between mean of quantitative parameters are marked by various letters in the same raw are significant (P≤ 0.05). ** Mean there is a highly significant differences (P≤ 0.01).

Table 3 -Average values (Means ± SE) of microbiological properties for sausage of Ostrich and Beef

Variables	Over All	Sausage		Sig.
		Ostrich	Beef	
E.coli (MPN/g)	2.50+0.33	0	5.00±0.47^{a}	**
Pseudomonas (cfu/g)	0	0	0	NS
Staphylococcus (cfu/g)	2.08×10^{3}±0.13	3.33×10^{2}±0.19^{b}	3.83×10^{3}±0.19^{a}	**
Total coli form (MPN/g)	17.67±0.65	7.33±0.92^{b}	28.00±0.92^{a}	**
Yeast and Mould (cfu/g)	1.00×10^{2}±0.34	0	2.00×10^{2}±0.49^{a}	**
Total viable count (cfu/g)	3.48×10^{6}±0.88	6.43×10^{4}±1.24^{b}	6.90×10^{6}±1.24^{a}	**

* The differences between mean of quantitative parameters are marked by various letters in the same raw are significant (P≤ 0.05). ** Mean there is a highly significant differences (P≤ 0.01). NS: non-significant

Table 4 - Salmonella presence in sausage from ostrich and beef

Detection of salmonella	Sausage			
	Ostrich		Beef	
	N	%	N	%
Positive	0	0	1	33.3
Negative	3	100	2	66.7
Total	3	100	3	100

The pseudo of sausage processed from ostrich and beef were zero (CFU/g), zero (CFU/g) respectively. There were no significant differences in Pseudomonas count observed among ostrich and beef sausage. The Staphylococcus of sausage processed from ostrich and beef were 3.33×10^{2} (CFU/g), 3.83×10^{3} (CFU/g) respectively. There were highly significant difference (P≤0.01) in Staphylococcus among ostrich and beef sausage. While beef sausage recorded the highest content of Staphylococcus while the lowest content of Staphylococcus was found in ostrich sausage. The findings were in line with Hamad (2010) who stated out that staphylococcus of beef sausage was (52%) when investigated prevalence of *Escherichia. Coli*, salmonella and *Staphylococcus aureus* and processed meat in Khartoum State, while Atef et al. (2015) were not detected staphylococcus in beef sausage when studied the effect of Arak Stems Extracts on chemical characteristics bacteria activity and sensory evaluation of beef sausage products. The total coli form of sausage processed from different type of meat (ostrich and beef) 7.33 (MN/g), 28 (MPN/g) respectively.There were significant differences (P≤0.01) in total coli form content among sausages. The highest content of total coli form was found to be in beef sausage. The results in the present study were agreement with Atef et al. (2015), who found that total coli form count in beef sausage was detected when studied the effect of arak stems extracts on chemical characteristics bacteria activity and sensory evaluation of beef sausage products.

The yeast and moulds of sausage processed from ostrich and beef were zero (CFU/g), 2×10^{2} (CFU/g) respectively. There were highly significant differences (P≤0.01) in yeast and moulds between (ostrich and beef) sausage. The highest yeast and moulds content was found in beef sausage, while was not found in ostrich sausage. The total viable count of bacteria for sausage processed from ostrich and beef were 6.43×10^{5} (CFU/g), 6.90×10^{7} (cFU/g) respectively. There were highly significant differences (P≤0.01) in total viable count among all type of sausage. The higher content of total viable count was found in beef sausage, while the lower content of total viable count was recorded in ostrich sausage.

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The findings of study were greater than Atef et al. (2015) who pointed out that the total coli from count in beef sausage was 6.25 cfu/g when studied the effect of arak stems extracts on chemical characteristics bacteria activity and sensory evaluation of beef sausage products. Table 4 showed that the detection of salmonella sausage processed from ostrich was 0(N)-0% positive and 3(N)-100% negative. While beef sausage was 1(N)-33.3% positive and 2(N)-66.7% negative. There was no significant differences found among detection of salmonella for all types of sausage. The findings were disagree with Atef et al. (2015), who found that detection of salmonella in beef sausage was not detected but was agreement with Hamad (2010), who observed salmonella of beef sausage was (20%).

CONCLUSION

The findings of this study revealed that, chemical composition for ostrich sausage was the highest percentage in crude protein, Ash and total energy expenditure of fat while beef sausage was the highest percentage in moisture. For microbiological load, beef sausage showed the highest count in all microorganisms which included in the study except total coli form and Pseudomonas were not detected in sausages. The salmonella was not detected in ostrich sausage, while it was detected in beef sausage.

Recommendation

- It is strongly recommended to use ostrich meat for sausage processing.
- Promotion programs are needed to make ostrich meat products more acceptable to consumers.
- Control of E.*coli* and other food-borne pathogens such as *Salmonella* and *Staphylococcus aureus* can be achieved.

Precautions should include adequate cooking and avoidance of cross contamination of cooked meat by contaminated equipment, water or infected food handlers.

DECLARATIONS

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Authors' Contribution

All authors contributed in research conduction, analyzing and writing, equally.

Conflict of interests

The authors declare that they have no competing interests.

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BIOMASS YIELD AND QUALITY OF FODDER FROM SELECTED VARIETIES OF LABLAB (*Lablab Purpureus* L) IN NANDI SOUTH SUB-COUNTY OF KENYA

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ABSTRACT: Low quality feeds is the main challenge ailing livestock production among the small-scale farmers in the tropics. Cheaper sources of alternative high quality fodder supplements are needed to improve livestock productivity. The objective of this study was to determine biomass yield and quality of fodder from selected lablab varieties. Eight lablab varieties namely, DL1002, Ngwara Nyeupe, Echo-Cream, Black-Rongai, Eldo-Kt-Cream, Eldo-Kt-Black1, Brown Rongai and Eldo-Kt-Black2 were established in three sites of Nandi south sub county, Kenya. Randomized complete block design was used at farm level with four replications per site. Data on biomass yield, chemical composition and in vitro-dry matter digestibility of the eight lablab forages was collected. Biomass yield differed significantly among the lablab varieties ranging from 5.6-12.6 t DM/ha across the three sites. Highest biomass yield was recorded for Brown Rongai (12.6 t DM/ha) and lowest with DL1002 (5.6 t DM/ha). Crude protein (CP) content varied significantly between varieties with sites ranging from 19.6-23.9 g/100g. Highest CP was recorded with Eldo-Kt-Cream and Black Rongai (23.9 g/100g and 23.7 g/100g) across the three sites. For all the varieties, Neutral detergent fibre (NDF) ranged from 44.4-48.6 g/100g, acid detergent fibre (ADF) 31.6-35.7 g/100g and acid detergent lignin (ADL) 9.0-11.9 g/100g across the three sites. Highest NDF was recorded with DL1002 (48.6 g/100g), ADF with Eldoret-Kitale-Black2 (35.9 g/100g) and acid detergent lignin with DL1002 (11.7 g/100g). *In vitro* dry matter digestibility (IVDMD) varied significantly between varieties and sites ranging from 67.6-75.7 g/100g between the varieties across the three sites. Eldo-Kt-cream and Black Rongai had the highest IVDMD (75.7 and 74.4 g/100g) across the three sites. Eldoret-Kitale-Cream and Black Rongai varieties had better dry matter yield, crude protein and low fibre fractions compared to the other varieties signifying their potential to be recommended as supplement to low quality fodder by small-scale farmers.

Keywords: Biomass yield, Digestibility, Feed, Fodder, Lablab.

INTRODUCTION

In Kenya, livestock contributes over 12% to the Growth Domestic Product (GDP) and accounts for 47% of Agricultural GDP (Kabubo-Mariara, 2008). The adequate provision of livestock feed is key to food security especially in the developing countries as animals are capable of converting low quality feedstuffs into high quality foods such as meat, milk and eggs (Amare et al., 2020). Sixty to seventy percent of livestock production costs has been attributed to feeds (Amare et al., 2020). Increase in livestock production by small-scale farmers will majorly rely on proper utilization of locally available feed resources to meet nutrient requirements (Bell et al., 2018). In Kenya, almost one-third of the small-scale farmers experience insufficient livestock feeds as the main challenge especially in dry seasons (Lukuyu et al., 2011). Most farmers feed their livestock on low quality feeds such as natural grass, maize stover, wheat straw, bean haulms and banana pseudo stems that are deficient in protein content (Abera and Berhanu, 2017; Redae and Tekle, 2020; Yiberkew et al., 2020). Production and use of fodder legumes is one of the cheaper ways of increasing both the quantity and quality of livestock feeds (Sharma et al., 2018).

Dolichos bean (*Lablab purpureus* L. Sweet), a vegetable crop of Asia and Africa origin (Bhardwaj and Hamama., 2019), was reported by Keerthi et al. (2015) as a good protein supplement for low quality animal feeds. This legume belongs to the family Fabaceae, sub family Faboideae, tribe phaseoleae and sub-tribe Phaseolineae (Gupta et al., 2017). The legume grows fast and can easily provide fodder within three months after planting (ILRI, 2013). It can produce approximately 4.5-20.1 tones DM/ha depending on soil fertility and rainfall distribution (Abera and Berhanu, 2017). The whole plant contain protein content of 13-24.5% varying from variety to variety (Heuzé et al., 2014; Bhardwaj and Hamama, 2019) comparable with Lucerne (15%), Clover legume (20%) and Fresh faba bean (14-20%) CP (Roy et al., 2016). It is a widely cultivated, highly drought resistant legume vegetable crop that can be grown in tropics and subtropics where soil fertility is low to be used as human food, forage and as a cover crop for soil conservation (Kumar, 2017; Bhardwaj and Hamama., 2019).

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Kumar et al. (2018) observed that the use of less costly and easily available indigenous feed resources such as legume forages as opposed to commercial feeds had great ability to enhance livestock productivity. This study therefore aimed at evaluating the biomass yield and quality of varieties of *Lablab purpureus* as a supplement to low quality fodder.

MATERIALS AND METHODS

Description of the study site

The study was conducted in Nandi south Sub-County in the Rift Valley region of Kenya. The altitude ranges from 1,400 m along the border with Nyando district to 2,400 m ASL in the highlands. The common soil texture type is loam and clay. Temperatures range from 15 to 26 °C and rainfall between 1200-2000 mm p.a. It has two rainy seasons; the long rains between March and June and the short rains between October and early December and the dry season occurs from late December to March (Onyango et al., 2016). Three sites with different climatic conditions and soil fertility were selected within Nandi South: Koibem site with temperature of 18 °C, high fertile soils with nitrogen content of 0.38% and carbon of 3.91% with annual rainfall distribution of 2,000 mm p.a., Kiptaruswo site with temperature of 20 °C medium soil fertility with nitrogen content of 0.26%, carbon content of 1.87% with annual rainfall distribution of 1,700 mm p.a. Kapkarer site with temperature of 22 °C, low soil fertility with nitrogen content of 0.16% and carbon 1.44 % with annual rainfall distribution of 1,600 mm p.a., respectively (Omondi et al., 2011; Landon, 2014).

Experimental treatment and design

Treatments consisted of eight varieties of lablab (*L. purpureus)* namely: DL1002, Ngwara Nyeupe, Echo-Cream, Black Rongai, Eldo-Kt-Cream, Eldo-Kt-Black1, Brown Rongai and Eldo-Kt-Black2. Seeds were acquired from Kenya Agricultural Livestock and Research Organization (KALRO) Kitale and were tested for viability at KALRO Kibos prior to planting. Each Lablab variety was planted in 5×4 m plots in each of the four farms per site. The experiment was laid out in a Randomized Complete Block Design (RCBD) with each farm representing a block comprising of eight plots. Di-ammonium phosphate (DAP) fertilizer was applied at the rate of 30 Kg DAP/ha mixed with the soil before seeds were sown. Lablab seeds were sown at the onset of rains at a rate of 30kg seeds/ha with a spacing of 45 cm between rows and 30 cm between plants and two seeds per hill. Weeding was done twice at an interval of 21 days of emergence. Data taken included biomass yield, crude protein content, neutral detergent fibre (NDF), acid detergent fibre (NDF) and acid detergent lignin (ADL) and in vitro dry matter digestibility (IVDMD).

Determination of biomass yield

At 50% flowering, a fresh sample was harvested for each variety by randomly cutting the plants 5 cm above the ground from each plot to make approximately 1 kg for each variety per plot. The harvested materials were accurately weighed, sealed in polythene bags for oven drying at 60 °C. The rest of the plants within each plot were harvested by cutting at 5 cm above the ground. They were placed into gunny bags and weighed using a 100 kg dial scale graduated to the nearest 1 kg to obtain the fresh biomass yield per plot. After drying the fresh samples in an oven of 60 °C to a constant weight, the new weight was recorded and the samples were ground using a Wiley mill standard model No. 3 with sieve of 0.5 mm. The dry matter content was then determined by drying in an oven at 105 °C for 5hrs. Subsequently, the biomass yield (dry matter) per ha was estimated through extrapolation from the plot size by multiplying the wet weight obtained per plot by the percentage dry matter to get dry matter yield per unit area .

Determination of nutrient composition

Dried milled samples were analyzed for crude protein content following the procedure of AOAC (1995). Neutral detergent fibre (NDF), acid detergent fibre (NDF) and acid detergent lignin (ADL) were determined using the method of Van Soest et al. (1991). The two-stage in vitro dry matter digestibility was determined following the procedure of Tilley and Terry (1963).

Data analysis

The data on biomass yield per unit area, crude protein, fibre fractions and *in vitro*-dry matter digestibility for different *L purpureus* varieties were subjected to Analysis of variance (ANOVA) for variation between varieties (both within and between sites). The significant means were separated using Tukey's statistical test at a significant level of 5%.

RESULTS AND DISCUSSION

Biomass yield of different *Lablab purpureus* varieties

Dry matter yield of the lablab varieties differed significantly ($P<0.05$) both within and between sites except in Kapkarer site (Table 1). Within Kiptaruswo, highest dry matter yield was recorded with variety Brown Rongai and lowest with Echo-Cream. In Koibem high dry matter yield was observed with variety Brown Rongai while Eldoret-Kitale-Cream recorded lowest dry matter yield. Between sites, Echo-Cream variety recorded the highest dry matter yield in Koibem compared with Kapkarer and Kiptaruswo. Eldo-Kt-Cream recorded the highest dry matter yield in Kapkarer compared to Kiptaruswo and Koibem. The rest of the varieties had no significant differences in dry matter yields between the sites.

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Low dry matter yield was observed for early flowering varieties (40-45 days after emergence) as these varieties did not exhibit bushy growth characteristics, had fewer branches thus minimal foliage. The dry matter yields of lablab varieties within this study were within 4.5-9.6 t DM/ha reported earlier by Amole et al. (2013); Bowen et al. (2018) and Tulu et al. (2018). However the yields were higher than 4 t DM/ha reported by Hassan et al. (2014). The differences in dry matter yield in this study could be attributed to genetic variations among the lablab varieties (Amole et al., 2013; Tulu et al., 2018) or difference in rainfall distribution and soil fertility within the three sites (Omondi, et al., 2011; Kebede et al., 2016).

The between site variations could be attributed to environmental variability, variations in soil moisture content, the initial plant populations per plot and soil types as these factors were reported by Hassan et al. (2014) and Kebede et al. (2016) to cause dry matter yield fluctuations in lablab. Different soil characteristics between the three sites was reported by Omondi et al. (2011) as sandy loam soils in Kapkarer and clay soils in Kiptaruswo and Koibem, this might have also caused variation in dry matter yields of lablab between sites. In an earlier study, Landon (2014) reported that Koibem site had high, Kiptaruswo medium and Kapkarer low fertile soils. As such, the high biomass yields per unit area in Koibem could be attributed to high fertile soils that supported robust growth of lablab. This could also mean that each of the lablab varieties has different environmental adaptations in which they can derive well compared to others (Tulu et al., 2018). However, the dry matter yields obtained in this study were within the range 5-14 t DM/ha that was reported by Hassan et al. (2014) as the satisfactory dry matter yield for good forage legume.

Table 1 - Dry matter yields of *Lablab Purpureus* varieties in the study area

Sites / Lablab varieties	Kapkarer	Kiptaruswo	Koibem	Mean	SE
Ton DM /ha					
Black-Rongai	8.2	6.8^{abx}	8.9^{bcx}	8.0^{b}	1.5
Brown Rongai	11.9	10.5^{ax}	15.4^{ax}	12.6^{a}	2.9
DL1002	6.1	6.0^{abx}	4.8^{cx}	5.6^{b}	0.7
Echo-Cream	5.0	3.9^{by}	12.1^{abx}	7.0^{b}	1.3
Eldo-Kt-Black1	7.6	5.6^{bx}	6.4^{bcx}	6.5^{b}	0.9
Eldo-Kt Black2	7.2	7.7^{abx}	5.6^{cx}	6.8^{b}	1.2
Eldo-Kt-Cream	9.5	5.2^{by}	4.4^{cy}	6.3^{b}	1.2
Ngwara Nyeupe	6.0	5.5^{bx}	8.7^{bcx}	6.8^{b}	1.7
Mean	7.7^{a}	6.4^{a}	8.3^{a}	7.5	
SE	1.87	1.0	1.3	0.97	
LSD	5.5	2.95	3.82	2.73	
P Value	0.263^{ns}	0.007**	<.001**	<.001**	

Eldo: Eldoret; Kt: Kitale; DL: Dry land variety; ^{abc}Values with different superscripts within column are significantly different; ^{xyz}Values with different superscripts within row are significantly different (*P<0.05; **P<0.001; ns: Non-significant.

Crude protein of different varieties of *Lablab purpureus*

The crude protein content of different lablab varieties within and between sites is shown in Table 2. Crude protein of lablab varieties varied significantly (P<0.05) both within and between the sites. The mean crude protein was significantly higher with lablabs that were grown in Koibem compared to those that were grown in Kapkarer and Kiptaruswo. Kiptaruswo, highest CP was recorded with DL1002 while lowest with Brown Rongai. In Koibem site, high CP content was recorded with variety Eldo-Kt-Cream and Black Rongai while lowest with Echo-Cream. There was significant interaction of CP content between lablab varieties and sites. The CP content of similar varieties was higher in Koibem compared with same in Kapkarer and Kiptaruswo. Across the three sites, Eldo-Kt-Cream and Black-Rongai had the highest mean crude protein content while Brown Rongai and Echo-Cream variety had the lowest.

In general, crude protein content of the eight lablab varieties in this study ranged from 18.0 to 26.5 g/100g. This was in agreement with results from other studies (18-23 g/100g) when the whole lablab plant was harvested at 50% flowering (Heuzé et al., 2014; Tulu et al., 2018; Bhardwaj and Hamama, 2019). Lower CP content of the whole lablab plant has also been reported in various studies ranging from 15-17 g/100g (Mbuthia et al., 2003; Njarui et al., 2003 and Mapiye et al., 2007). Crude protein is one of the indices that is usually used to assess the feed quality (Liu et al., 2019). The variability of CP content in lablab varieties in this study enables us to choose the suitable variety that can be used as a supplement for low quality forges (Geleti et al., 2013). The variations in crude protein content among the varieties of lablab and across the sites in this study and with those by others was attributed to difference in genotypes and soil fertility (Geleti et al., 2013; Kebede et al., 2016; Tulu et al., 2018; Washaya et al., 2018). Additionally, the low crude protein content in Brown Rongai variety could be explained by observation by Washaya et al. (2018) that biomass yield of legume forages were negatively correlated with their quality. According to Kazemi et al. (2012), legume forages with CP above 19% were considered of high quality while those with <8% CP were regarded as of low quality hence cannot be used as a supplement for low quality fodder. Tulu et al. (2018) reported that most herbaceous legumes >15% CP could support

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growth and lactation of dairy animals. This indicates that the CP values of all lablab varieties in this study met the threshold of being used as a supplement to low quality feeds for lactating animals (Geleti et al., 2013).

Table 2 - Crude protein content (g/100g) of *Lablab Purpureus* varieties

Lablab varieties \ Sites	Kapkarer	Kiptaruswo	Koibem	Mean	SE
Crude Protein content					
Black-Rongai	21.2	24.1^{abxy}	25.6^{abx}	23.7^{a}	1.0
Brown Rongai	19.3	18.0^{cx}	21.4^{bcx}	19.6^{b}	1.1
DL1002	18.7	26.3^{ax}	23.1^{abcx}	22.7^{ab}	0.9
Echo-Cream	19.6	20.5^{bcx}	20.0^{cx}	20.0^{b}	1.3
Eldo-Kt-Black1	18.6	22.6^{abx}	24.7^{abcx}	22.0^{ab}	0.9
Eldo-Kt-Black2	22.0	20.9^{bcy}	25.8^{abx}	22.9^{ab}	1.1
Eldo-Kt-Cream	21.7	23.6^{abxy}	26.5^{ax}	23.9^{a}	1.2
Ngwara Nyeupe	20.6	20.2^{bcx}	22.5^{abcx}	21.1^{ab}	1.0
Mean	20.2^{a}	22.0^{b}	23.7^{c}	22.0	
SE	1.02	0.92	1.04	0.79	
LSD	3.01	2.7	3.06	2.21	
P Value	0.159^{ns}	<.001**	0.002**	<.001**	

Eldo: Eldoret; Kt: Kitale; DL: Dry land variety; ^{abc}Values with different superscripts within column are significantly different; ^{xyz}Values with different superscripts within row are significantly different (*P<0.05; **P<0.001; ns: Non-significant.

Fibre fraction of eight selected *Lablab purpureus* varieties

The fibre fractions of the eight *Lablab purpureus* varieties are shown in Table 3. There were significant differences (P<0.05) between fibre fractions of the lablab varieties both within and between the sites. Higher neutral detergent fibre (NDF) was recorded for lablab varieties harvested in Kapkarer site (mean=51.4 g/100g) and lowest in those established in Koibem (mean=42.9 g/100g). In Kapkarer and Kiptaruswo, all the varieties had similar NDF content unlike in Koibem site. In Koibem, DL1002 variety had the highest NDF while lowest recorded with varieties; Echo Cream and Black-Rongai respectively. Between the sites, NDF content of all the lablab varieties varied significantly except DL1002 which had similar NDF content within the three sites. Across the three sites, DL1002 had the highest NDF while Black-Rongai variety had the lowest. Eldo-Kt-Black2 recorded the highest acid detergent fibre (ADF) in Kapkarer site while Eldo-Kt-Black1 had the lowest. In Kiptaruswo, highest ADF was recorded with Eldo-Kt-Cream and lowest with Black Rongai. Within Koibem site, high ADF was recorded with Black Rongai variety and lowest with Eldo-Kt-Cream. The ADF content of the varieties varied significantly between sites except DL1002 and Ngwara Nyeupe. Across the three sites, highest ADF was recorded with Eldo-Kt-Black2 with Eldoret-Kitale-Cream having the lowest. Within Kapkarer site, Eldo-Kt-Cream had the highest acid detergent lignin (ADL) and Eldo-Kt-Black1 the lowest. In Kiptaruswo, no significant variation was observed in ADL content among the varieties of lablab. Within Koibem, Eldo-Kt-Black1 had the highest ADL and Echo Cream the lowest. Between the sites, Eldo-Kt-Black1 recorded the highest ADL in Koibem and Kiptaruswo than in Kapkarer. Eldo-Kt-Cream had the highest ADL in Kapkarer than in Koibem. In general, high ADL was observed for Eldo-Kt-Cream with Echo Cream having the lowest across the three sites.

The NDF content of the eight lablab varieties in this study ranged from 44.4 g/100g and 48.6 g/100g across the three sites. Similarly, NDF content of the whole lablab plant 45-48 g/100g has been reported by others (Heuzé et al., 2014; Bhardwaj and Hamama, 2019). However, lower NDF content of 40.09 and 39.0 g/100g for the whole lablab plant was reported by Ahmad et al. (2000) and Mbuthia et al. (2003). The whole fiber fraction of fodder is confined in the NDF or cell walls and it gives the best approximation of the entire fiber content of a feed. The neutral detergent fibre is negatively associated with feed intake with its increase in forage reduces feed consumption (Garcia et al., 2003). The study by Geleti et al. (2013) and Yiberkew et al. (2020) indicated that, forage plants with NDF content less than 45% were regarded as of high quality while those between 45-65% were medium quality and above 65% as low quality forage. Profile of NDF content from the eight lablab varieties in this study were of high quality in Koibem sites while Kapkarer and Kiptaruswo were of medium quality. Therefore, all the lablab varieties in this study had acceptable NDF content for ruminant animals.

The variation in NDF content between varieties of lablab in this study could also be due to genetic variations among the lablab varieties that were used (Amole et al., 2013). The between site variations in NDF content could be due to variation in soil fertility between the sites. Studies by Turk (2010); Kebede et al. (2016) and Yiberkew et al. (2020) indicated that, fertile soils increases the forage dry matter yield and crude protein content which results in reduction of NDF and ADF content by accumulation of more foliage than structural features. This observation is consistent with the results of NDF content of lablab varieties in in this study/ Koibem site, with higher fertile soils, had lablab with lower NDF content compared to Kapkarer site.

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Table 3 - Fibre fractions (%) of various *Lablab purpureus* varieties grown in the study area

Sites / Varieties of lablab	% Neutral detergent fibre (NDF)					% Acid detergent fibre (ADF)					% Acid detergent lignin				
	Kap	Kip	Koi	Mean	SE	Kap	Kip	Koi	Mean	SE	Kap	Kip	Koi	Mean	SE
Black Rongai	49.5	43.2	40.4^{cy}	44.4^{b}	0.97	33.0^{bcy}	28.7^{cy}	45.4^{ax}	35.7^{ab}	1.8	9.8^{abx}	9.1	9.8^{abx}	9.6^{ab}	1.2
Brown Rongai	53.2	49.4	41.1^{cy}	47.9^{ab}	0.94	34.4^{bx}	36.2^{abx}	28.3^{bcy}	33.0^{ab}	1.2	9.3^{abx}	9.7	11.2^{abx}	10.1^{ab}	0.8
DL1002	52.2	46.7	47.0^{ax}	48.6^{a}	2.13	33.8^{bcx}	30.1^{cx}	34.4^{bx}	32.8^{ab}	1.9	12.7^{ax}	11.6	10.8^{abx}	11.7^{a}	1.2
Echo Cream	49.5	45.1	40.4^{cy}	45.0^{ab}	1.23	32.4^{bcy}	29.4^{dy}	41.8^{ax}	34.5^{ab}	1.1	9.6^{abx}	9.5	8.0^{bx}	9.0^{b}	0.7
Eldo-Kt-Black1	50.0	43.8	45.9^{abxy}	46.6^{ab}	1.66	28.5^{cy}	34.6^{abx}	33.4^{bx}	32.2^{ab}	1.3	7.3^{by}	10.0	12.1^{ax}	9.8^{ab}	0.8
Eldo-Kt-Black2	55.5	46.1	43.3^{bcy}	48.3^{ab}	1.99	42.1^{ax}	35.1^{aby}	30.4^{bcz}	35.9^{a}	0.8	11.3^{abx}	12.2	11.1^{abx}	11.5^{a}	0.4
Eldo-Kt-Cream	53.0	46.1	41.4^{cz}	46.8^{ab}	0.55	33.4^{bcx}	37.0^{ax}	24.4^{cy}	31.6^{b}	0.9	13.3^{ax}	12.2	10.1^{aby}	11.9^{a}	0.9
Ngwara Nyeupe	48.5	44.6	43.6^{abcy}	45.5^{ab}	0.93	33.5^{bcx}	31.2^{bx}	33.7^{bx}	32.8^{ab}	1.0	11.1^{abx}	10.6	10.1^{abx}	10.6^{ab}	0.6
Mean	51.4^{a}	45.6^{b}	42.9^{c}	46.6		33.9^{a}	32.8^{a}	34^{a}	33.6		10.6^{a}	10.6^{a}	10.4^{a}	10.5	
SE	1.62	1.85	0.77	1.48		1.17	1.92	1.49	1.51		0.88	1.05	0.74	0.88	
LSD	4.76	5.44	2.26	4.19		3.44	5.64	4.4	4.26		2.58	3.08	2.19	2.47	
P Value	0.072^{ns}	0.393^{ns}	<.001**	<.001**		<.001**	0.026*	<.001**	<.001**		0.002**	0.251 ns	0.033*	0.001**	

Kap: Kapkarer; Kip: Kiptaruswo; Koi: Koibem; Eldo: Eldoret; Kt: Kitale; DL: Dry land Variety; ^{abc}Values with different superscripts within column are significantly different; ^{xyz}Values with different superscripts within row are significantly different (*P<0.05; **P<0.001); ns: Non-significant

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The range in ADF content for all the varieties of lablab in this study was 31.6 g/100g to 35.7 g/100g. These values were in agreement with 33-35 g/100g ADF reported earlier in the whole lablab forage by several authors (Heuzé et al., in 2014; Washaya et al., 2018; Bhardwaj and Hamama, 2019). Lower ADF content of 25-28 g/100g in lablab fodder was also reported by Ahmad et al. (2000) and Mbuthia et al. (2003). Acid detergent fibre is an indication of the degree of cellulose and lignin in forage. It is negatively associated with general digestibility; high ADF feed is less digestible (Garcia et al., 2003). The difference in ADF content among the varieties of lablab could be due to genetic variation among the varieties. According to Kazemi et al. (2012), legume forage with less than 31% ADF content was regarded as of high quality, while that greater than 55% NDF was regarded as poor quality. Conversely, Geleti et al. (2013) and Yiberkew et al. (2020) noted that, forage plant with less than 40% ADF was regarded as of high quality while those with greater than 40% as of poor quality. The ADF values of all the lablab varieties in this study therefore were of high quality.

The lignin content of the lablab varieties in this study ranged from 9.0 to 11.9 g/100g. These were within the range of 6.3 to 13.7 g/100g reported in the whole lablab plant by several authors (Ahmad et al., 2000; Heuzé et al., 2014; Bhardwaj and Hamama, 2019). Lignin is a polymer fraction of the plant cell walls that offers rigidity and mechanical support to plants and is not digestible by animal enzymes. It increases with plant maturity and was reported to be greater when the same plant species are established under warm weather conditions (Garcia et al., 2003). The figures of ADL content for the eight lablab varieties were close and some beyond the maximum level of 10% that is required to limit forage use by ruminant animals in this study (Geleti et al., 2013; Yiberkew et al., 2020). The difference in lignin content among the varieties of lablab in this study might be due to genetic differences between the varieties and different climatic conditions within the three sites (Amole et al., 2013; Kebede et al., 2016).

In vitro dry matter digestibility of different lablab varieties

In vitro-dry matter digestibility (IVDMD) of the different *L. purpureus* varieties is shown in Table 4. The IVDMD differed significantly (P<0.05) among the varieties both within and between the sites apart from in Kapkarer. Of the three sites, highest mean dry matter digestibility was recorded for varieties that were established at Koibem site compared to those established in Kapkarer and Kiptaruswo. At Kiptaruswo site high dry matter digestibility was recorded for Eldo-Kt-Cream and Black-Rongai variety with the lowest for Eldo-Kt-Black2. In Koibem, high dry matter digestibility was recorded with Eldo-Kt-Cream and Black Rongai variety, while lowest was DL1002. Between the sites, all the varieties, except Eldo-Kt-Black1, varied significantly in IVDMD. Across the three sites, highest dry matter digestibility was recorded for Eldo-Kt-Cream and Black Rongai while lowest for DL1002, Brown Rongai and Ngwara Nyeupe.

Table 4 - *In vitro*-dry matter digestibility (%) of Lablab varieties grown in different sites

Sites / Lablab varieties	Kapkarer	Kiptaruswo	Koibem	Mean	SE
***In vitro*-dry matter digestibility**					
Black Rongai	68.6	75.3^{ay}	79.4^{abx}	74.4^{ab}	0.64
Brown Rongai	69.4	60.5^{bcz}	73.7^{bx}	67.8^{b}	0.97
DL1002	69.2	72.1^{ax}	61.4^{cy}	67.6^{b}	1.67
Echo Cream	68.6	71.7^{ay}	76.5^{abx}	72.3^{ab}	1.09
Eldo-Kt-Black1	68.3	71.2^{ax}	74.8^{abx}	71.4^{ab}	2.13
Eldo-Kt-Black2	72.5	59.6^{cy}	75.8^{abx}	69.3^{ab}	2.15
Eldo-Kt-Cream	70.3	76.3^{axy}	80.4^{ax}	75.7^{a}	1.54
Ngwara Nyeupe	69.6	70.6^{abx}	65.4^{cy}	68.5^{b}	1.36
Mean	69.6^{b}	69.7^{b}	73.4^{a}	70.9	
SE	1.58	2.22	1.2	1.62	
LSD	4.66	6.52	3.53	4.54	
P Value	0.663^{ns}	<.001**	<.001**	0.002**	

Eldo- Eldoret, Kt- Kitale, DL- Dry land Variety, ^{abc}Values with different superscripts within column are significantly different ^{xyz}Values with different superscripts within row are significantly different (*P<0.05; **P<0.001), ns:Non-significant.

The *in vitro*-dry matter digestibility of various lablab that were tested in this study ranged from 67.6 to 75.7 g/100g across the three sites. Mapiye et al. (2007) reported the *in vitro*-dry matter digestibility of the whole lablab plant to range from 55 to 76 g/100g in agreement with those obtained in this study. However Ahmad et al. (2000) and Tulu et al. (2018) reported a lower dry matter digestibility of lablab foliage as 39.7-50.9 g/100g. The variation in *IVDMD* in this study with other authors could be due to difference in varieties of lablab that were used and their fibre content (Meale et al., 2012; Tulu et al., 2018). The fibre fractions in legume forages has been the major challenge in their adequate utilization in animal nutrition (Washaya et al., 2018). The high fibre fractions in a forage leads to low dry matter intake and digestibility as opposed to lower fibre fractions (Washaya et al., 2018). Varieties with high dry matter digestibility in this study such as Eldo-Kt-Cream and Black-Rongai were associated with high crude protein that increased microbial activities for organic

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matter breakdown low NDF, ADF and ADL content. Varieties with low dry matter digestibility such as DL1002, Ngwara Nyeupe and Brown Rongai were associated with high NDF, ADF and ADL and late maturity. The difference in dry matter digestibility could also be attributed to genetic differences among the lablab varieties and the climatic conditions of the location where plants within cool environment are associated with high dry matter digestibility as opposed to warm environment (Baloyi et al., 2013).

CONCLUSION

From the study, it can be concluded that biomass yield and nutrient content varied with lablab variety and production site. According present findings, variety Brown-Rongai was suitable for all sites and can be recommended as a supplement to low quality animal fodder.

DECLARATIONS

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Author's contribution

Due to site variation with biomass yields of lablab varieties, Kapkarer farmers can grow Brown-Rongai, Eldoret-Kitale-Cream and Black-Rongai, all authors has similar attempts in all process of conduction and writing of present study.

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Conflict of interests

The author declare that there is no conflict of interests on this work

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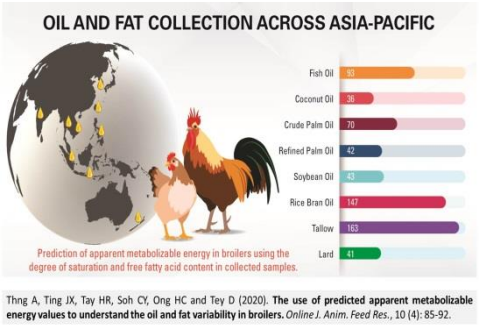

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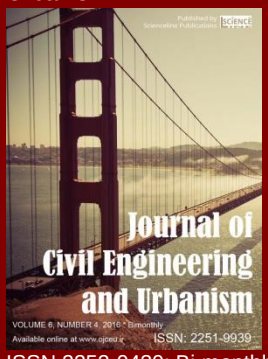

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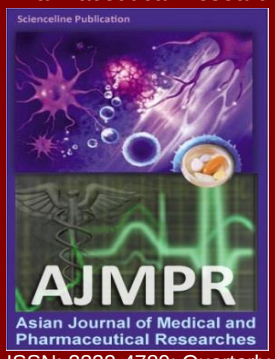

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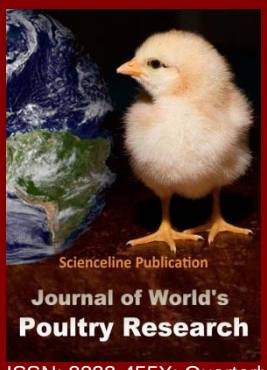

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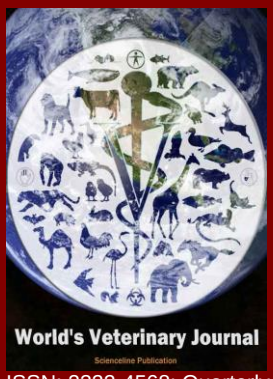

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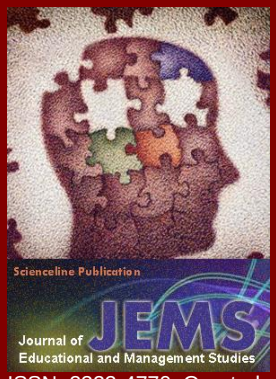

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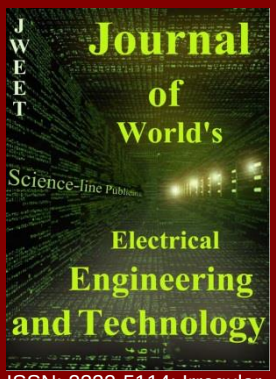

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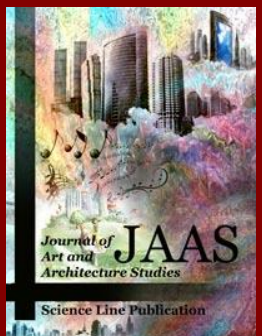

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