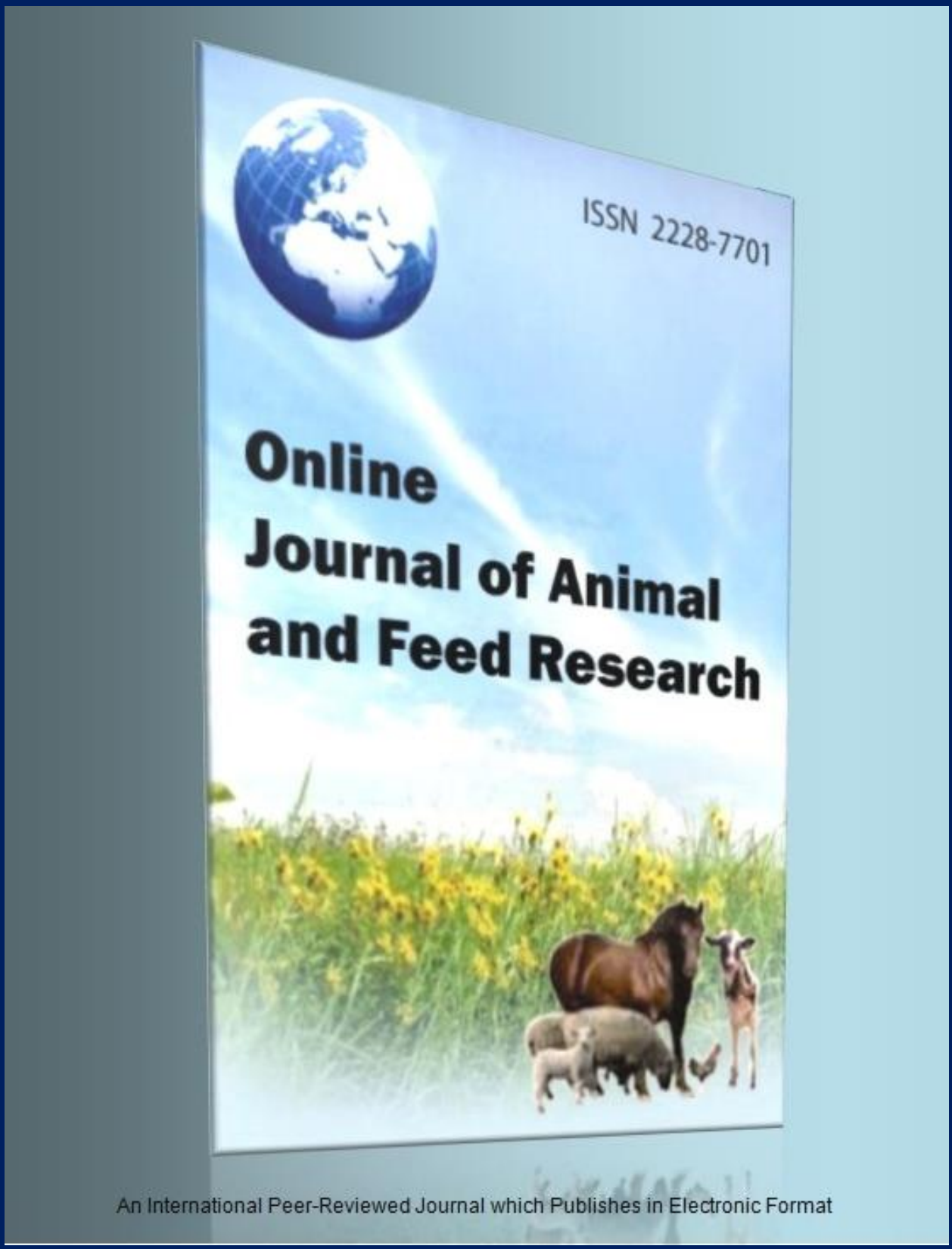
ONLINE JOURNAL OF ANIMAL AND FEED RESEARCH



Volume 9, Issue 2, March 2019

Online J. Anim. Feed Res., 9 (2): March 25, 2019

Editorial Team

Join OJAFR Team

As an international journal we are always striving to add diversity to our editorial board and operations staff. Applicants who have previous experience relevant to the position may be considered for more senior positions (Section Editor, SE) within OJAFR. All other members must begin as Deputy Section Editors (DSE) before progressing on to more senior roles. Editor and editorial board members do not receive any remuneration. These positions are voluntary.
If you are currently an undergraduate, M.Sc. or Ph.D. student at university and interested in working for OJAFR, please fill out the application form below. Once your filled application form is submitted, the board will review your credentials and notify you within a week of an opportunity to membership in editorial board.
If you are Ph.D., assistant or associate editors, distinguished professor, scholars or publisher of a reputed university, please rank the mentioned positions in order of your preference. Please send us a copy of your CV or ORCID ID or briefly discuss any leadership positions and other experiences you have had that are relevant to applied Animal and Feed Researches or publications. This includes courses you have taken, editing, publishing, web design, layout design, and event planning.
If you would like to represent the OJAFR at your university, join our volunteer staff today! OJAFR representatives assist students at their university to submit their work to the OJAFR. You can also, registered as a member of OJAFR for subsequent contacts by email and or invitation for a honorary reviewing articles.

Download OJAFR Application Form

Archive

Volume 9 (2); March 25, 2019

Research Paper

Anatomical and histological study of the rabbit spleen development in the postnatal period in Algeria.

Eddine RD, Amine FM, Farida BA, Khadidja Bd.

Online J. Anim. Feed Res., 9(2): 44-50, 2019; pii: S222877011900007-9

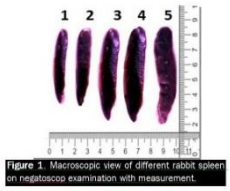


Figure 1. Macroscopic view of different rabbit spleen on negatoscop examination with measurement.

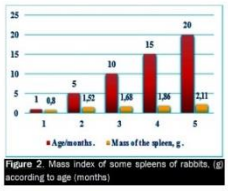


Figure 2. Mass index of some spleens of rabbits. (g) according to age (months)

To cite this paper: Eddine RD, Amine FM, Farida BA, Khadidja Bd (2019). Anatomical and histological study of the rabbit spleen development in the postnatal period in Algeria. *Online J. Anim. Feed Res.*, 9(2): 44-50. www.ojafr.ir

Abstract
Our research describes the morphological and histological changes of the rabbit's spleen; local breed of eastern Algeria. In general, the spleen has a rectangular shape with a triangular section, rounded edges, more or less ridged, the surface is smooth, the color is brownish red becomes dark red with age, the capsule and the parietal surface are shiny and smooth. His organ measurement and mass parameters are given. Particular emphasis placed on its microstructure; especially on changes occur during development. Parenchyma histological composition analysis performed using statistical methods. Twenty-month-old rabbits known to have follicles with mantle strongly developed, parenchyma's quantitative analysis components showed significant changes.
Keywords: Capsule, Follicles, Lymphoid, Parenchyma, Rabbit, Spleen.

[Full text-PDF]

Research Paper

Detection of antibodies against Newcastle and infectious bursal disease on chicken in north Gondar zone, Ethiopia.

Birhan Mas., Birhan Mal., Tesfaye Sh, and Tariku A.

Online J. Anim. Feed Res., 9(2): 51-58, 2019; pii: S222877011900008-9

Abstract
Infectious Bursal (IBD) and Newcastle diseases (NCD) are an acute highly contagious and destructive illness of chickens that occur almost any time of the year. In this study, we aimed to detect antibodies against NCD and IBD The study was compared a compressive investigation on the Ab detection local and exotic breed with different serological technique of Indirect-ELISA and Haemagglutination inhibition (HIT) test. In this study, a cross-sectional study design was used and applied to collect 384 serum samples from chickens which were selected by simple random sampling. Serum samples were collected from three different districts which were Dembiya, Dabark and Metema. The serum sample collections were also considered different parameters like sex, age, breed, infection, and vaccination during the data collection time. The results showed that the overall Ab detection of IBD and NCD Virus were 74.4% and 79.6% respectively. Epidemiological status of both IBD and NCD Virus of each districts were indicted Metema (72.2%, 86.0%), Dembiya (89.7%, 92.4%) and Dabark (67.2%, 69.1%) respectively. In conclusion, these research findings extend the presence of detectable antibodies to NCD and IBD in chickens, in the three districts of North Gondar Zone. As it is known Chickens' might have been high detectable antibodies if they are either susceptible to the pathogenic or are vaccinated.

Keywords: Newcastle, infectious bursal, Antibody, Chickens, Indirect-ELISA test and Haemagglutination inhibition.

[Full text-PDF]

Research Paper

Effects of low temperature upon hatchability and chick quality of Ross-308 broiler breeder eggs during transportation.

Hussain A, Bilal M, Habib F, Gola BA, Muhammad P, Kaker A, Yousaf A, Khalil R.

Online J. Anim. Feed Res., 9(2): 59-67, 2019; pii: S222877011900009-9

Abstract
In current experiment n=20,000 Ross-308 broiler breeder eggs collected from 33 week old flocks were subjected to cold stress during transportation. Eggs were allocated to 4 temperature groups (treatments): 1.5 °C, 2 °C, 3.5 °C, 5 °C, and a control group, 20 °C. Each treatment had 4,000 eggs, and data were

analyzed in a completely randomized design. The results of this study showed that cold stress had a significant effect on percentage of egg weight loss ($P < 0.001$), and minimal egg weight loss occurred in the control group. The percentage of exploders and early hatched chicks and chick weight were higher in the below 3°C temperature treatment than the other groups ($P < 0.01$). Cold stress had a significant effect on chick length, hatchability, and the hatching of fertile eggs ($P < 0.001$). The effects of cold stress on chick yield and body weight uniformity were significant ($P < 0.01$). The effect of cold stress on hatchery by product efficiency was significant ($P < 0.001$), but did not affect fertility. Cold stress also had significant effects on early (1-8 days), middle (9-17 days), and late mortality (20-21 days); total embryo mortality; and exposed brain. Ectopic viscera was significant ($P < 0.001$), and most mortality was observed in below 4 °C treatments. Total percentages of malpositions and deformity ($P < 0.001$) and egg contamination at 1-9 days (first stage) and 10-21 days (second phase) were affected by cold stress ($P < 0.001$). Cold stress also had a significant impact on the number of cull chicks; percent of string navel, button navel, total string, and button; omphalitis; full body cavity; red hocks; dehydration; dirty chickens; and stubby down. Cold stress affects performance during incubation and overall chick quality.
Keywords: Cold Stress, Egg Transportation, Hatchability, Chick Quality, Malposition And Deformity, Culls, Egg Weight Loss, Embryo Mortality And Contamination

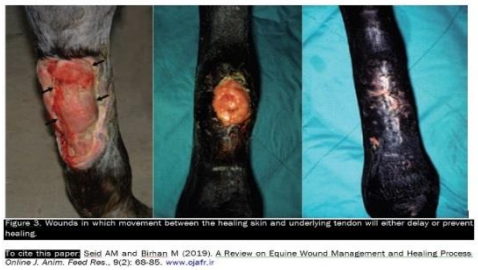
[Full text-PDF]

Review

A Review on Equine Wound Management and Healing Process.

Seid AM and Birhan M.

Online J. Anim. Feed Res., 9(2): 68-85, 2019; pii: S222877011800010-9



Abstract
Skin lacerations and other traumatic injuries of the integument are frequently seen in equine practices and range from relatively minor cuts to severe, potentially debilitating injuries. The early stages of wound healing (inflammatory or debridement stage) that are clean or clean contaminated are the best candidates for primary or delayed primary closure. Wounds that are in the later stages of healing or are contaminated or infected heal best by second intention. Management is dictated by the nature and size of the wound, the area of the body on which the wound occurs, and several aspects of wound healing. The age of the wound, integrity of the local blood supply, degree of contamination, location of the injury, skin loss, and local tissue damage must all be considered when deciding on the most appropriate method for managing a particular wound. In addition to biologic factors, the physical size of equine patients and the environment in which they are kept present unique management challenges not encountered in the treatment of soft tissue injuries in other species. Appropriate wound care is always a balance between improving the wound environment and harming the cells that are integral to the healing process. Consequently, the veterinarian must carefully weigh the benefits and the detriments of any particular action, not only for the immediate results but also for the long-term healing process. Understanding the principles and limitations of reconstructive procedures, adhering to the basic principles of equine wound management, and providing appropriate postoperative care all contribute to a successful outcome.
Keywords: Debridement, Equine, Inflammation, Management, Wound

[Full text-PDF]

Research Paper

In vitro digestibility and *in situ* degradability of sugarcane bagasse treated with urea as energy source in total mixed ration for goat.

Elmnan B,A.Atta and Ismeal R,A.

S222877011800011-9

Abstract
This study was carried out to investigate the effect of replacing sorghum grains (SG) by sugarcane bagasse treated with 5% urea (SCBU) as energy source in total mixed ration. *In vitro* dry matter digestibility (IVDMD) and *in situ* DM degradability were adopted to conduct the experiment. In order to meet the nutrient requirement of goat, the following four iso-caloric and iso-nitrogenous rations were formulated: A= 0% SCBU (control); B= 2.6% SCBU replaced 10% SG; C= 5.2% SCBU replaced 20% SG; and D= 7.8% SCBU replaced 30% SG. The IVDMD was significantly ($P \leq 0.05$) affected by dietary level of SCBU which was decreased gradually with increasing SCBU levels in examined rations. Ration A gained the highest value of IVDMD (88.50%) followed by ration B (84 ,(%C (78 (%and D (74.50%). An *in situ* DM degradability results showed that the readily soluble fraction (a), slowly fermented material (b) and the potential degradability (PD (were significantly ($P \leq 0.05$) higher for ration A than rations B, C and D .Although DM digestibility and degradability of rations B, C and D were decreased with progress level of SCBU in the rations, the values obtained from present study might be considered within the range of moderate to high level of digestibility and degradability especially ration B which replaced 10% of SG. More research is needed to study the effect of replacing SG by SCBU regarding the nutrients intake, animal performance in an *in vivo* experiment and to determine its economic feasibility.
Keywords: Sorghum Grains, Bagasse, Urea, Energy, Total Mixed Ration

[Full text-PDF]

Research Paper

Chemical composition and sensory evaluation of meat of *Tswana* hens fed different protein sources: Mopane worm, Bambara groundnut and Morama bean.

Manyeula F, Tsopito Ch-M, Mogotsi K, Nsoso Sh-J, Kamau JM and Moreki JC.

Online J. Anim. Feed Res., 9(2): 92-102, 2019; pii: S222877011800012-9

Abstract
This study investigated the chemical composition and sensory evaluation of meat of *Tswana* hens fed diets containing mopane worm (*Imbrasia belina,* Westwood) or Bambara groundnut (*Vigna subterranea* (L) Verde) or morama bean (*Tylosema esculentum,* Burchell A. Schreiber) as different protein sources under intensive management system. Sixty Tswana hens (25 weeks old) were bought from a local farmer and reared up to 38 weeks of age on diets containing *T. esculentum* or *V. subterranea* or *I. belina* and a commercial layer diet as a control. Experimental diets met the nutritional composition of control diet. Whole thighs, drumsticks obtained from the carcasses at week 28, 33 and 38 were deboned. The thigh, drumstick meat and bone were analysed for phosphorus (P), calcium (Ca) and potassium (K) using AOAC methods. Furthermore, boiled 2 x 5 cm portions of drumsticks, breasts and thighs were organoleptically assessed for flavour, odour, juiciness, tenderness and firmness by untrained panelists (15 males and 15 females) using a 5-point Likert scale. Bone P and Ca were high in all treatment diets at 28 weeks compared to 38 weeks of age. The meat DM, CP, P, Ca and K and bone P, Ca and K contents were not significantly affected by treatment diets. Organoleptic quality from all treatment groups and across different ages received moderate (3.52) to good rating (4.13). In conclusion, *Imbrasia belina* (Mopane worm) or *Vigna subterranea* (Bambara groundnut) or *Tylosema esculentum* (Morama bean) can replace soybean meal in *Tswana* hens without compromising meat and bone chemical composition and also organoleptic qualities.

Keywords: Crude protein, Minerals, Organoleptic Attributes, Protein Sources, *Tswana* hens

[Full text-PDF]

Research Paper

Comparison of farm formulated and commercial winter mineral lick on nutrients utilization and sheep performance.

Kuleile N and Molapo S.

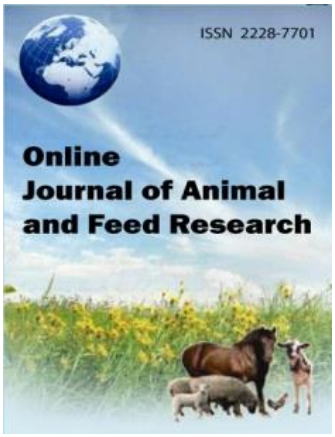
Online J. Anim. Feed Res., 9(2): 103-109, 2019; pii: S222877011800013-9

Abstract
This A completely randomized study with three dietary treatments was undertaken at the National University of Lesotho Faculty of Agriculture farm. The three treatments were made up of control which had no access to winter mineral lick supplement and two treated groups made up of farm formulated and commercial winter lick. All animals had access to basal diet in the form of Teff [*Eragrostis tef* (Zucc.) Trotter]. The main objectives of the study were to assess the influence of supplementary feeding using winter mineral lick on sheep performance and on the utilization of teff. Also to compare both farm formulated and commercial winter mineral lick on the performance of sheep and utilization o ىEragrostis tef*.* A total of 30 yearling sheep of similar body weight were used in this study and were housed in pairs. Dietary treatments and water were offered ad libitum while basal diet was pre-weighed on daily basis. The findings of this study indicated that dietary treatments had a significant ($P < 0.05$) influence on feed intake, live weight and weight gain while there were no significant ($P > 0.05$) difference on feed conversion ratios. The control group of animals had poor production performance than treated groups and animals were losing weight. Nutrients digestibility were statistically different amongst all the treatments whereby supplement groups had better nutrient utilization than the control group. Mean comparison test revealed that there were no significant difference between farm formulated and commercial winter mineral lick on production and digestibility parameters. Economic benefit analysis indicated that by using farm formulated winter mineral lick farmers can save up to 36% of feed costs. To further reduce the costs of farm formulated lick, there is need to consider non-conventional feeds such as brewery by-products in the formulation of lick.
Keywords: Digestibility, *Eragrostis Tef*, Farm Formulated, Nutrient Utilization, Winter Mineral Lick

[Full text-PDF]

Previous issue Next issue Archive

Online Journal of Animal and Feed Research



ISSN: 2228-7701

Frequency: Bimonthly

Current Issue: 2019, Vol: 9, Issue: 2 (March)

Publisher: SCIENCELINE

Online Journal of Animal and Feed Research is an international peer-reviewed journal, publishes the full text of original scientific researches, reviews, and case reports in all fields of animal and feed sciences, bimonthly and freely on the ınternet ...view full aims and scope

www.ojafr.ir

» OJAFR indexed/covered by NLM/PubMed, CABI, CAS, AGRICOLA, DOAJ, Ulrich's™, GALE, ICV 2015 = 71.65), Worldcat, EZB, TOCs ...details

» Open access full-text articles is available beginning with Volume 1, Issue 1.

» Full texts and XML articles are available in ISC-RICeST and DOAJ.

» This journal is in compliance with Budapest Open Access Initiative and International Committee of Medical Journal Editors' Recommendations.

» High visibility of articles over the internet.

Editorial Offices:
Atatürk University, Erzurum 25100, Turkey
University of Manitoba, Winnipeg, Manitoba R3T 2N2, Canada
University of Maragheh, East Azerbaijan, Maragheh 55136, Iran
Homepage: www.science-line.com
Phone: +98 914 420 7713 (Iran); +90 538 770 8824 (Turkey); +1 204 8982464 (Canada)
Emails:
administrator@science-line.com
saeid.azar@atauni.edu.tr

2019 SCIENCELINE

Online Journal of Animal and Feed Research

Volume 9, Issue 2: 44-50; March 25, 2019 ISSN 2228-7701

AN ANATOMICAL AND HISTOLOGICAL STUDY OF THE RABBIT SPLEEN DEVELOPMENT IN THE POSTNATAL PERIOD IN ALGERIA

Djallal Eddine RAHMOUN ✉, Mohamed Amine FARES, Farida BOUZEBDA-AFRI, Khadidja BEN DRISS

Laboratory of Animal Production, Biotechnology and Health, Departement of Veterinary Science, Institut of Agronomic and Veterinary Science Taoura, University of Souk Ahras, Algeria

Supporting Information

ABSTRACT: **Our research describes the morphological and histological changes of the rabbit's spleen; local breed of eastern Algeria. In general, the spleen has a rectangular shape with a triangular section, rounded edges, more or less ridged, the surface is smooth, the color is brownish red becomes dark red with age, the capsule and the parietal surface are shiny and smooth. His organ measurement and mass parameters are given. Particular emphasis placed on its microstructure; especially on changes occur during development. Parenchyma histological composition analysis performed using statistical methods. Twenty-month-old rabbits known to have follicles with mantle strongly developed, parenchyma's quantitative analysis components showed significant changes.**

Keywords: **Capsule, Follicles, Lymphoid, Parenchyma, Rabbit, Spleen.**

ORIGINAL ARTICLE

PII: S222877011900007-9

Received: January 10, 2019

Revised: February 20, 2019

INTRODUCTION

The mammalian spleen works like a blood deposit. Since birth, red blood cells and lymphocytes are present, also in the postnatal period, the spleen is an organ of lymphopoiesis and protects the body against cells and genetically foreign substances, and also participates in humoral immune responses (Cesta 2006), also, it's a parenchymal organ, unpaired, and participates in the metabolism, especially iron and protein (Tiron et al., 2008). In the field of digestion, the spleen has a physical and chemical role, the blood thickens in the spleen and the elements formed separate to some extent the plasma in which they are suspended (Udroiu et al., 2017). The morphology of the animal and human spleen was carried out by researchers (Udroiu et al., 2017). The morphometry of the white pulp of the spleen in Yorkshire pig has been studied by Shringi et al. (2017).

Spleen studies in goats have been conducted by Hassan et al. (2018). In addition, extensive studies on the human spleen have been conducted by Nerschbach et al. (2016). Like other animals, white and red pulps are distinguished in the spleen of rabbits. The white pulp of the spleen is mainly populated by the corresponding differentiated lymphocytes. The areas of the white pulp include arterial vessels surrounded mainly small lymphocytes (lymph tissue T-dependent), with clear boundaries. Furthermore, in the white pulp of the spleen rabbits, there are lymphoid follicles grouped by lymphoid cells. They are surrounded by a well-defined connective tissue. These lymphoid follicles belong to lymphoid tissue B-dependent (Thomas et al., 1967). The red pulp as white has clear boundaries. Lymphoid follicles are strongly developed according to age and have weak germinal centers. In addition, the white pulp has a distinctive feature: The B-dependent zone is formed after the T-dependent zone and has previously undergone an age-related physiological involution, cited by Nerschbach et al. (2016). Scientific work is devoted to the in-depth study of the normal morphology of different compartments of the spleen, in different species of animals. However, data from the scientific literature regarding the changes in age-related anatomical and histological structure of the rabbit spleen during ontogeny are insufficient.

The objective of this study is to describe the anatomical and histological differences in development of spleen in rabbits during the postnatal period. This work will be of interest for future research in veterinary medicine as well as in biological and medical sciences.

MATERIALS AND METHODS

The ethical approval is not necessary for such type of study. We have used the spleen of rabbits were presented for post-mortem examination. We dissected 50 healthy male rabbits, aged 01-05-10-15 and 20 months, local breed of

To cite this paper: Rahmoun DE, Fares MA, Bouzebda-Afri F, Ben Driss Kh (2019). Anatomical and histological study of the rabbit spleen development in the postnatal period in Algeria. *Online J. Anim. Feed Res.*, 9(2): 44-50. www.ojafr.ir

the Souk-Ahras region in Algeria, divided into five age groups according to table 1, whose live weight ranged from 89 to 4200 g. The rabbits were weighed before beginning the experiment in the laboratory of Animal Production, Biotechnology and Health, University of Mohamed Cherif Messaâdia, Souk Ahras, Algeria.

A macro-morphometric study (weight gain using a "Tehniprot-WTW" scale with an error point of 0.002 mg) and the measurement of the length and width dimensions of the organs with a GOCT17435-72 ruler set to 1mm. The collected spleens were subjected to a macroscopic and histological study. The following technique was adopted to prepare histological slide; Tissues obtained from rabbits were fixed in 10% formalin for 24 hours and then underwent successive passes through the various compartments; dehydrated in increasing concentration of ethanol, then cleared in xylene and finally soaked in paraffin. The residence time of the fragments in the automate is 24 hours. The blocks were then cut to a thickness of 5 µm with a microtome. The sections were placed into a flotation bath at 37° C. Then, they were placed on the slides and dried on a hot plate. The sections were stained by hematoxylin and eosin to determine the general structures of rabbit spleen and Wiegert hematoxylin and picrofushin (Van Gieson) to study the structural features of the connective tissue stroma. Other frozen sections impregnated with silver nitrate to determine the characteristics of reticular stromal areas of the parenchyma of the spleen. Relative area of tissue components determined by the method of "exact calculation" using S test systems (Dunaievska, 2018) and using optical microscopes Leica DM2000 LED. The photomicrographs made with the digital camera Sony ILCE-6000. Data obtained from our morphometric and histological studies have been performed with the Excel 2016 program.

Table 1 - Rabbit spleen morphometry and mass according to age

Age group, months	1	5	10	15	20
Weight of the animal, g	890±100	1350±100	2400±100	3900±100	4200±100
Mass of the spleen, g	0,8±0,062	1,52±0,026	1,68±0,0688	1,86±0,05	2,11±0,12
Length of the spleen, mm	42,88±0,3	45,8±0,354	61,214±0,95	71,2±1,19	65,28±0,97
Width of the spleen, mm	5,28±0,17	6,66±0,18	7,7±0,19	9,98±0,18	11,3±0,19

RESULTS AND DISCUSSION

After desiccation, the spleen was not directly visible, hidden by the left edge of the stomach, and its axis is in border with the great gastric curvature, in contact with the left costal wall by the external face and in contact with the stomach and the left kidney by the internal surface.

Studies have shown that rabbit spleen may have the following form: oval elongated, with slightly sharp edges, an irregular shape, "baguette", with a process caudate to the back end with a pointed rear end (Figure 1). The oval shape of the spleen has rounded dorsal and ventral ends, the upper and lower edges are smooth. Shaped "drumstick", the dorsal edge of the spleen is transferred and becomes "head" of the "drumstick". The size of this part is nine, six wide and three millimeters thick. Ventral end wider than two millimeters dorsal grows up to eleven millimeters. Smooth sharp edge, wavy and blunt edge.

When the spleen is oval with a caudate process, its dorsal end is six mm in width and one and a half millimeters in length. Next is the three mm extension of the original. The shoot is at a distance of four mm from the dorsal extremity. Its dimensions are four in length, two in width, one and a half millimeters thick, and four millimeters wider than the dorsal in oval ventral extremity. The sharp and blunt edges are smooth. In the case of an irregular shape, the body has a modified rear end of seven centimeters wide, one and a half millimeters thick. The ventral extremity is slightly pointed, with a width equal to six mm. The dull edge is uniform, sharp in the region of the ventral extremity is also smooth, but undulating at the dorsal extremity. In the form with a pointed dorsal extremity, it initially has a width of three, extremity mm wide, still extends three millimeters. Its length is 15 mm, its thickness is five. The ventral end is wider and reaches six mm. Smooth dull edges, wavy sharp edge, the spleen is elongated, with slightly pointed margins, its dorsal extremity has a breadth equal to five mm and a thickness of a one mm and a half, and there is an increase after five mm. The ventral end is seven mm wide and one and a half millimeters thick. Blunt and sharp edges are smooth. In most cases, there is an elongated spleen with some sharp edges. Different forms of the spleen can be explained by the fact that the growth and development of the organ are irregular, and the shape and size of the adjacent organs and the pressure they exert on the formation of internal organs the abdominal cavity.

Morphological changes observed in the hue of the rabbit spleen, the spleen of rabbits aged one month, the hue is red-brown, becomes darker in rabbits which are twenty months old, the lateral edges lose their hue and become less distinct than in the center, this feature was observed in these rabbits with a strong curvature of the arc, a low position and a left shift. The study of the mass of the spleen of the rabbit has revealed a gradual increase of 0.8 g for

To cite this paper: Rahmoun DE, Fares MA, Bouzebda-Afri F, Ben Driss Kh (2019). Anatomical and histological study of the rabbit spleen development in the postnatal period in Algeria. *Online J. Anim. Feed Res.*, 9(2): 44-50. www.ojafr.ir

spleens of older rabbits a month and this increase is proportional, reaching 2.11 g for rats aged rabbits 20 months (Figure 2).

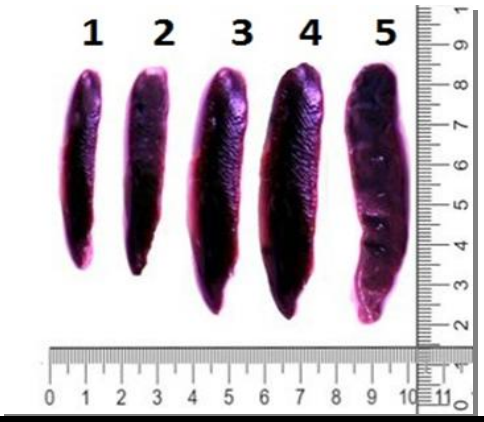


Figure 1. Macroscopic view of different rabbit spleen on negatoscop examination with measurement.

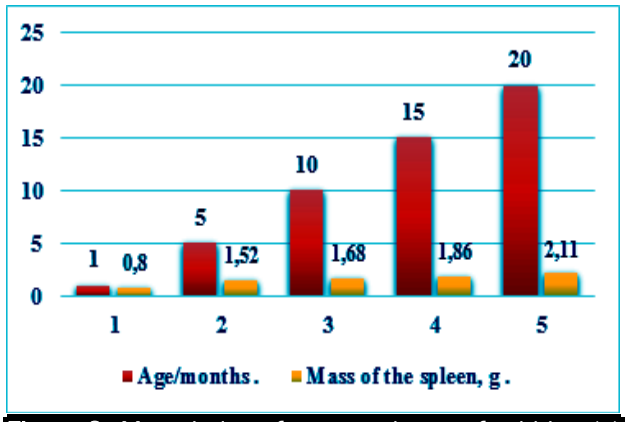


Figure 2. Mass index of some spleens of rabbits, (g) according to age (months)

The length reaches 42.88 mm for the spleen of the animals aged one month and 65.28 mm for the animals aged 20 months, the width was 5.28 mm found in the spleen of rabbits by one month and the value maximum of 11.3 mm for the spleen of 20-month-old rabbits (Figure 3). The length of this portion is nine millimeters, six wide and three millimeters thick. The ventral end is larger than the two-millimeter dorsal. At a distance of seven millimeters from the ventral end, the organ extends to eleven millimeters.

Morphological examination of the spleen of rabbits aged fifteen months revealed a thicker lateral surface than the underneath with three zones of vascular irrigation, wrapped in a thin layer of connective tissue; in particular a fat layer surrounding the end of the spleen, this has also been proved by the researcher (Hristov et al., 2006), the proximal part freely forms a distension curved in height, concerning the spleen of the first group, the fatty layer is minimal and occupies the apical layer of the layer of connective tissue lining the entire splenic zone from the turgor in the ventral part, there has more fixations equal to nine for this age, while the spleen of rabbits aged twenty months, from which the number of supplements reaches 14 according to (Lalić et al., 2018). Thus, the rabbit spleen is characterized by polymorphism and morpho-functional changes that, no doubt, should reflect increasing or decreasing functional processes in the respective developmental periods and affecting the immune system of the animal. The texture, size, weight, color and shape of the spleen vary significantly depending on the duration of its functional activity, age, species and race of the animal (Eberlova et al., 2017).

Stereo-microscopic examination (MBS) revealed rabbit's spleen consists of stroma and parenchyma, stroma formed by capsule and trabeculae that penetrate inside parenchyma. It has been noted that capsule consists of two layers: external connective tissue (elastic) and internal (muscular), allowing the organ to change size and maintain a significant increase in its volume, this was mentioned by Nawal et al. (2018). The muscle layer consists of polymorphic myocytes (Figure 4).

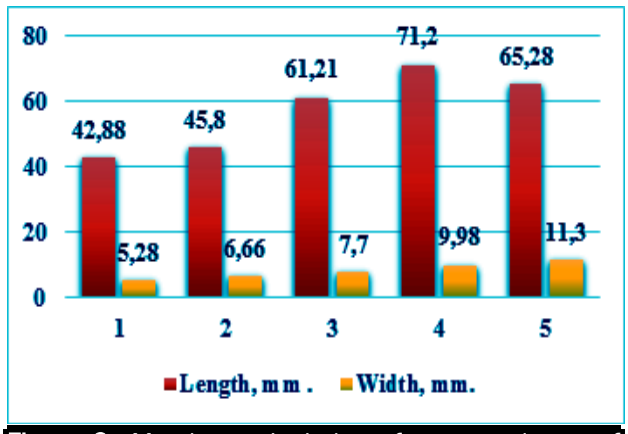


Figure 3. Morphometric index of some spleens of rabbits, (mm) according to the age (month).

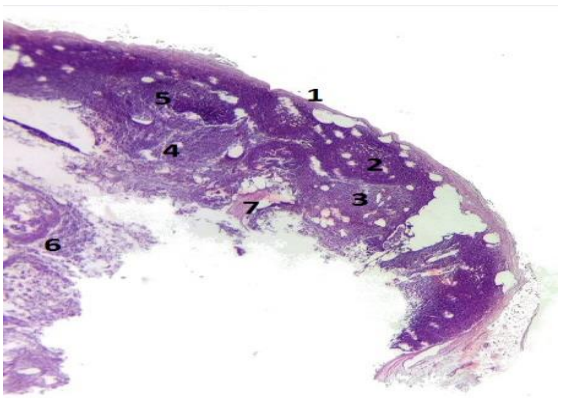


Figure 4. Spleen of one-year-old rabbit. hematoxylin-eosin stain, X 5.7. 1- capsule; 2- red pulp; 3- white pulp. 4 –cortical zone; 5- Cortico-medullary zones; 6- medulla zone; 7- Trabecula.

To cite this paper: Rahmoun DE, Fares MA, Bouzebda-Afri F, Ben Driss Kh (2019). Anatomical and histological study of the rabbit spleen development in the postnatal period in Algeria. *Online J. Anim. Feed Res.*, 9(2): 44-50. www.ojafr.ir

At high magnification, it has been observed that red pulp is composed of splenic sinusoidal capillaries and cellular cords. Splenic sinusoids are limited by batch simple epithelium, resting on abasement membrane around sinusoids discontinuously, sinusoidal capillaries contain many blood cells (Figure 5), what has been identified in other spleens by the authors (Medaglia et al., 2017).

It was found that surface of the red pulp is almost identical in spleen of the 1st and 2nd group of age, within maximum is 78.1%, this zone is minimal in the spleen of rabbits aged 10 and 15 months (Figure 5), equal almost 70,78%, deduced that surface of the red pulp decreases with age. These results were consistent with another author research (Fukuta et al., 1969). Concerning white pulp, it should be noted that the surface of the white pulp decreases in the parenchyma of the spleens of the rabbits aged ten months (Figure 6), while 11.56% for spleens of rabbits aged five months. This increase reaches the maximum of 16.04% in the spleen of rabbits aged 20 months. Questions to note about the decrease in spleen value in five-month-old rabbits, probably due to a decrease in antigenic activation.

Capsule trend increase varies from spleen of rabbits aged one month's 10.44% to the spleen of rabbits aged 15 months 14.67%, due to increase in spleen's volume. Also, it has been found that white pulp has several compartments such as peri-arteriolar lymphoid sheaths (P.A.L.S), whose spleen of rabbits aged one month, had a maximum level of P.A.L.S with value of 9.35 ± 0.55%, while the spleen of rabbits aged 20 months, the minimum value was 1.63 ± 0.35%.

Follicles quantitative study shows that rate is almost equal for 2 follicles types (primary and secondary) equal 16.17% 17.38% in spleen of rabbits aged five months. This value also identical in spleen of rabbits aged 15 months 34.45% and 35.82%. It was found that follicles activation is stable probably due to the non-immunization of animals with vaccines, contrary, this finding was not established for rabbits immunized by vaccines, this increase presents change point for secondary lymphoid follicle. Scrutiny optical microscope revealed that mantle secondary lymphoid follicle in marginal areas stand out easily, this was proven by Pinkus et al. (1986), the border zone is transition zone between white and red pulp, also mentioned in research, on mice spleen.

Spleen connective tissue network is highlighted by silver staining, forming precipitates around reticulin fibers. Spleen capsule is crossed by blood vessels, which enter into organ via spans. White and red pulps are clearly visible. Lymphoid follicle has well-defined germinal center and crown (Figure 7). Central artery is also easy to see during this preparation. Reticulin fibers form extensive network through spleen, anchored on capsule and on spans. White pulp comprises lymphoid follicles complex of spleen that fulfill a protective function and produce a larger mass of lymphocytes in the blood. The red pulp has small round growths.

Each site crossed by artery running along periphery, similar research has been demonstrated by other research. Lymphoid tissue silver nitrate impregnation was put in evidence as blood supply to red pulp, which comes from penicillar arteries which give rise arterioles, including endothelial cells and smooth muscle cells well visible in center elucidated also.

Impregnation with silver nitrate, was observed that venous sinuses can be found throughout red pulp, directly adjacent to edge region bordered by endothelial cells loose network lie on basal membrane which sandwiched between endothelial cells and red pulp reticular fibers. In white pulp (Figure 8), it is subdivided into PALS, this research and results conformed with data to Gill et al. (2017), lymphoid follicles and marginal zone. Central arterioles surrounded by PALS in red pulp, composed of lymphocytes and reticular fibers concentric layers and reticular flattened cells.

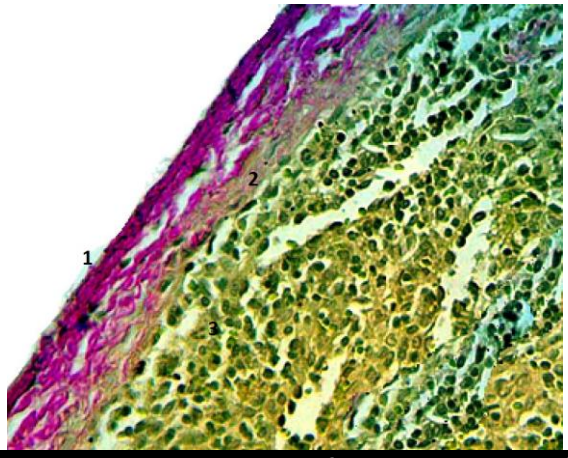


Figure 5. Histological section of rabbit spleen aged 15 months. Van Gieson stain, X40. 1 -layer of connective tissue (elastic). 2 -muscular layers. 3 -splenic parenchyma.

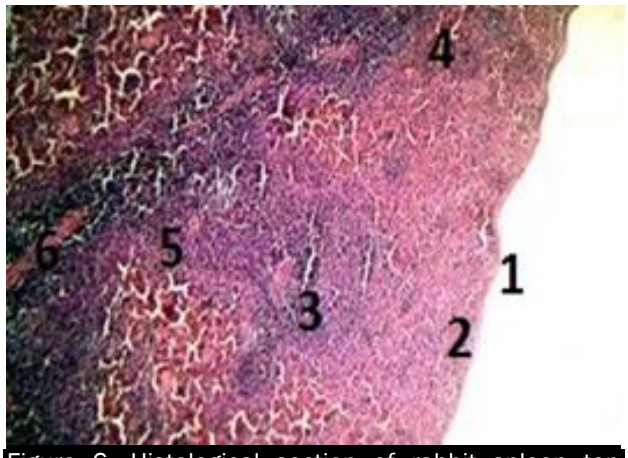


Figure 6. Histological section of rabbit spleen ten months old. Hematoxylin and eosin, × 200: 1 capsule; 2 – subcapsular trabecula; 3 – red pulp; 4 - lymphatic vessels; 5 – white pulp.

To cite this paper: Rahmoun DE, Fares MA, Bouzebda-Afri F, Ben Driss Kh (2019). Anatomical and histological study of the rabbit spleen development in the postnatal period in Algeria. *Online J. Anim. Feed Res.*, 9(2): 44-50. www.ojafr.ir

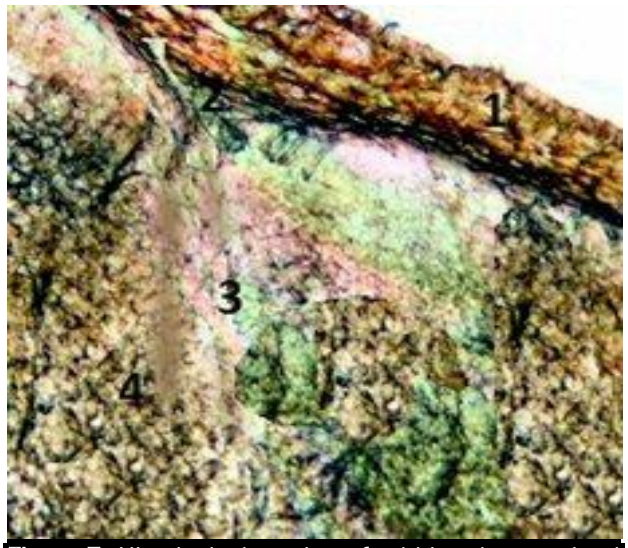


Figure 7. Histological section of rabbit spleen stained with silver nitrate **X 200. 1 - capsule 2 - trabeculae; 3 - pulp artery; 4 – layer of reticular fibers**

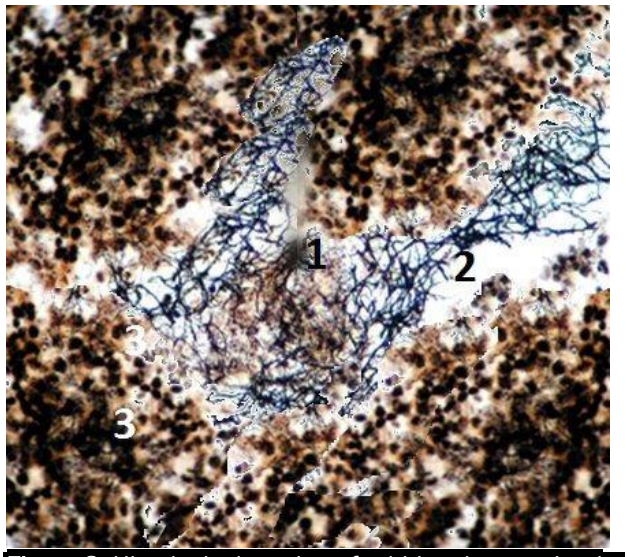


Figure 8. Histological section of rabbit spleen stained with silver nitrate, X400. 1 - lumen of the vessel; 2 - reticular fibers; 3 – lymphocytes.

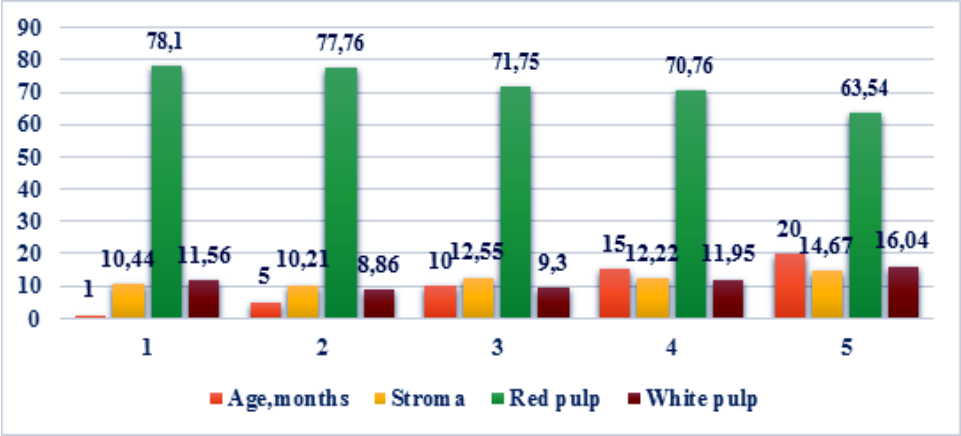


Figure 9. Histogram, rabbit spleen compositions (%) according to age (months). Graph4. White pulp compositions (%) according to age (months).

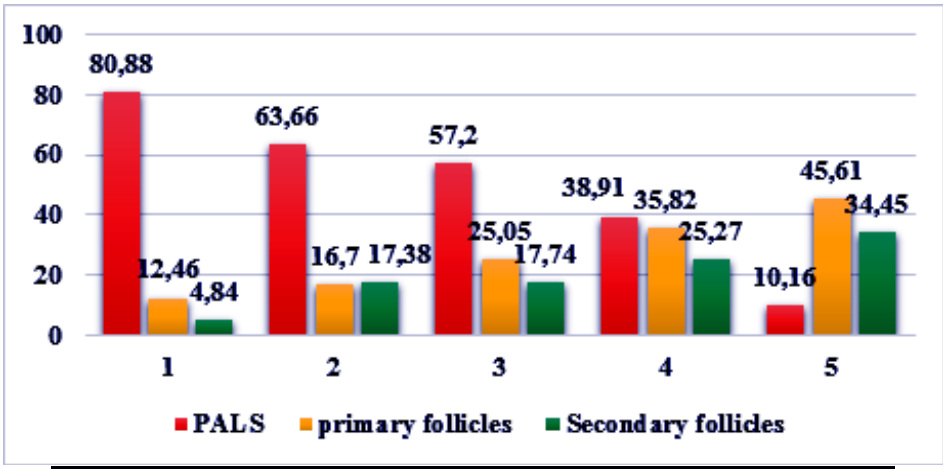


Figure 10. Graph4. White pulp compositions (%) according to age (months).

CONCLUSION

Capsule surface and trabecular meshwork increased by age and form frame for white and red pulp, spleen white pulp shows peri-arteriolar lymphatic system (PALS) and lymphoid follicles, marginal zone separates red to white pulp

To cite this paper: Rahmoun DE, Fares MA, Bouzebda-Afri F, Ben Driss Kh (2019). Anatomical and histological study of the rabbit spleen development in the postnatal period in Algeria. *Online J. Anim. Feed Res.*, 9(2): 44-50. www.ojafr.ir

could also be clearly demarcated. In the first group lymphoid tissue still growing, in deformed state, smooth muscle tissue predominates structurally. Twenty months ago, increase in lymphoid follicles percentage and increased smooth muscle tissue. can concluded with animal's age, produces spleen morphological changes. Appearance of reactive centers indicates primary nodules converted into secondary lymphoid follicles, occurs at new locations along blood vessels. Tissue components histogenesis from rabbits spleen during postnatal ontogeny characterized by primary lymphoid follicles intensive development, in context of corresponding significant changes in quantitative parameters other components first stage (up to 30 days), during growth, main parenchyma components tend to decrease, except primary lymphoid follicles in second phase (up to 10 months), accompanied respectively an increase of stroma relative area; Finally, third stage (up to 20 months) absence of significant changes in relationship between stromal and parenchymal components of the organ.

In conclusion according to statistical analysis, 1st group PALS area reaches maximum value and decreases in first age; leaving place for primary and secondary follicular lymphoid.

DECLARATIONS

Corresponding Author

E-mail: deddine44@hotmail.com ; ORCID: 0000-0001-6723-4491

Author's contribution

All authors contributed equally to this work.

Competing interests

The authors declare that they have no competing interests.

Acknowledgements

The authors wish to thank the team of the histology laboratory of the faculty of veterinary sciences of Taoura, University of Souk-ahras Algeria, for their support and help throughout the research period.

REFERENCES

Cesta MF (2006). Normal structure, function, and histology of the spleen, Toxicologic pathology, 34 (5): 455-465.

Dunaievska O (2018). Anatomical and Morphometric Criteria of Spleen in Matured Gallus gallus, forma domestica L., Columbia livia G., Coturnix coturnix L. Innovative Biosystems and Bioengineering, 2(4), 221-231.

Eberlova L, Liska V, Mirka H, Tonar Z, Haviar S, Svoboda M. and Mik, P (2017). The use of porcine corrosion casts for teaching human anatomy. A Fukuta nnals of Anatomy-Anatomischer Anzeiger, 213: 69-77.

Gill N, Nasir A, Douglin J, Pretterklieber B, Steinke H, Pretterklieber M and Cotofana S (2017). Accessory Spleen in the Greater Omentum: Embryology and Revisited Prevalence Rates. Cells Tissues Organs, 203(6): 374-378.

Hassan AA, Nossir HM, Soliman KZ El-Skeikh EM, and Konsowa M M (2018). Computed tomographic, laparoscopic and sectional anatomy of the liver and spleen in goats (capra hircus). Veterinary Medicine in-between Health & Economy (vmhe)–16-19 October 2018 55, no. 20-suppl (2018).

Hristov H, Kostov D and Vladova D (2006). Topographical anatomy of some abdominal organs in rabbits. Trakia Journal of Sciences, 4(3): 7-10.

Lalić IM, Bichele R, Repar A, Despotović SZ, Petričević S, Laan M and Milićević NM (2018). Lipopolysaccharide induces tumor necrosis factor receptor-1 independent relocation of lymphocytes from the red pulp of the mouse spleen. Annals of Anatomy-Anatomischer Anzeiger, 216: 125-134.

Medaglia C, Giladi A, Stoler-Barak L, De Giovanni M, Salame T M, Biram A, and Amit I (2017). Spatial reconstruction of immune niches by combining photoactivatable reporters and scRNA-seq. Science, 358(6370): 1622-1626.

Nawal AN and Maher MA (2018). Gross Anatomical, Radiographic and Ultra-structural Identification of Splenic Vasculature in some Ruminants (Camel, Buffalo Calf, Sheep and Goat). Int. J. Adv. Res. Biol. Sci, 5(2): 44-65.

Nerschbach V, Eberle N, Joetzke AE, Hoeinghaus R, Hungerbuehler S, Mischke R and Betz D (2016). Splenic and hepatic ultrasound and cytology in canine lymphoma: effects of findings on stage migration and assessment of prognosis. Veterinary and comparative oncology, 14, 82-94.Rehfeld, Anders, Malin Nylander, Kirstine Karnov (2017), The Immune System and the Lymphatic Organs. Compendium of Histology. Springer, Cham: 379-409.

Pinkus GS, Warhol MJ, O'connor EM, Etheridge CL and Fujiwara K (1986). Immunohistochemical localization of smooth muscle myosin in human spleen, lymph node, and other lymphoid tissues. Unique staining patterns in splenic white pulp and sinuses, lymphoid follicles, and certain vasculature, with ultrastructural correlations. The American journal of pathology, 123(3), 440.

Shringi, Nikhil, Rakesh Mathur, Kavita Rohlan, Vikas Kumar, and Subha Ganguly. "Morphometry of Spleen in White Yorkshire Pig (Sus scrofa)." Int. J. Pure App. Biosci 5, no. 4 (2017): 755-757.

Thomas CE (1967). An electron- and light-microscope study of sinus structure in perfused rabbit and dog spleens. American Journal of Anatomy, 120(3), 527-551.

To cite this paper: Rahmoun DE, Fares MA, Bouzebda-Afri F, Ben Driss Kh (2019). Anatomical and histological study of the rabbit spleen development in the postnatal period in Algeria. *Online J. Anim. Feed Res.*, 9(2): 44-50. www.ojafr.ir

2019 SCIENCELINE

Online Journal of Animal and Feed Research

Volume 9, Issue 2: 51-58; March 25, 2019

ISSN 2228-7701

DETECTION OF ANTIBODIES AGAINST NEWCASTLE AND INFECTIOUS BURSAL DISEASE ON CHICKEN IN NORTH GONDAR ZONE, ETHIOPIA

Mastewal BIRHAN[✉], Malede BIRHAN, Shimelis TESFAYE, and Amare TARIKU

[1]*College of Veterinary Medicine and Animal science, Department of Veterinary Paraclinical studies, University of Gondar, Gondar, Ethiopia*
[2]*College of Veterinary Medicine and Animal science, Department of Animal Nutrition, University of Gondar, Gondar, Ethiopia*
[3]*College of health science and medicine, Department of Human Nutrition, University of Gondar, Gondar, Ethiopia*

Correspondence should be addressed to Mastewal Birhan; maste675@gmail.com; ORCiD: 0000-0002-0984-5582

Supporting Information

ORIGINAL ARTICLE
PII: S222877011900008-9
Received: August 16, 2018
Accepted: December 19, 2018
Revised: February 30, 2019

ABSTRACT: **Infectious Bursal (IBD) and Newcastle diseases (NCD) are an acute highly contagious and destructive illness of chickens that occur almost any time of the year. In this study, we aimed to detect antibodies against NCD and IBD The study was compared a compressive investigation on the Ab detection local and exotic breed with different serological technique of Indirect-ELISA and Haemagglutination inhibition (HIT) test. In this study, a cross-sectional study design was used and applied to collect 384 serum samples from chickens which were selected by simple random sampling. Serum samples were collected from three different districts which were Dembiya, Dabark and Metema. The serum sample collections were also considered different parameters like sex, age, breed, infection, and vaccination during the data collection time. The results showed that the overall Ab detection of IBD and NCD Virus were 74.4% and 79.6% respectively. Epidemiological status of both IBD and NCD Virus of each districts were indicted Metema (72.2%, 86.0%), Dembiya (89.7%, 92.4%) and Dabark (67.2%, 69.1%) respectively. In conclusion, these research findings extend the presence of detectable antibodies to NCD and IBD in chickens, in the three districts of North Gondar Zone. As it is known Chickens' might have been high detectable antibodies if they are either susceptible to the pathogenic or are vaccinated.**

Keywords: **Newcastle, infectious bursal, Antibody, Chickens, Indirect-ELISA test and Haemagglutination inhibition.**

INTRODUCTION

Chicken production has a major role in the economy of developing countries and backyard production is particularly important to women (Wilson, 2010). Several programmes, in Ethiopia and elsewhere, have attempted to improve chicken production as a means to reduce poverty (Sambo et al., 2015).

Like away in the developing world, backyard poultry rearing is a common practice in rural Ethiopia. Village backyard poultry, characterized by traditional production methods and local breeds, represents 98% of the total Ethiopian poultry population of 38 million (Chaka et al., 2012).

Small and large-scale chicken farms are rapidly growing in Ethiopia. The chicken strains imported are temperate breeds that are less adapted to the heat stress and disease challenges in the country. Accompanying intensification of poultry farming, there is occurrence of epidemics of newly introduced diseases and/or epidemics of endemic diseases (Anebo et al., 2014). One of the diseases that are of growing concern in poultry is Infectious Bursal disease (Gumboro disease). As in this report a large scale occurrence of Infectious Bursal disease in the central part of Ethiopia with intensive and high-density juvenile farms (Zeleke et al., 2005).

Infectious bursal disease (IBD) is a highly contagious, immunosuppressive infection of immature chickens with a worldwide distribution (Negash et al., 2012). Two serotypes of IBD virus strains are described: 1 and 2. Serotype 2 strains are classified as a pathogenic, and serotype 1 strain, pathogenic to chickens, is classified into several patho-types, from mild to hyper virulent, according to their virulence (Chaka et al., 2012).

IBD infection results in lymphoid tissue depletion and the final destruction of the bursa, which is the predominant feature of its pathogenicity (Okwor et al., 2012). This virus may exacerbate infection with other etiologic agents and reduce the chicken's ability to respond to vaccination. Susceptibility and breed of flock, types of virus strains, inter-current primary and secondary pathogens, and environmental and management factors influence the economic impact of IBD (Jenbreie et al., 2012)

To cite this paper: Birhan Mas., Birhan Mal., Tesfaye Sh, and Tariku A (2019). Detection of antibodies against Newcastle and infectious bursal disease on chicken in north Gondar zone, Ethiopia. *Online J. Anim. Feed Res.*, 9(2): 51-58. www.ojafr.ir

The other disease of poultry is Newcastle disease (NCD) also known as Ranikhet disease (RD), locally known as Fungal, is caused by avian paramyxovirus serotype 1 (APMV-1) belonging to the family Paramyxoviridae, genus *Avulavirus* (Ezema et al., 2009). Newcastle disease virus (NCD) can be categorized into highly pathogenic (velogenic), intermediate (mesogenic), and less pathogenic (lentogenic) strains based on pathogenicity in chickens and are divided in two clades (class I and class II) (Ali et al., 2004).

The epidemiology and control of NCD and IBD has been extensively studied and documented in commercial poultry systems, but has been poorly documented in village poultry (Awan et al., 1994). The large differences in management between commercial and village poultry prohibit the transfer of epidemiological data and control programmes of NCD and IBD from the commercial sector to the village environment (Yongolo et al., 2002). In developing countries these vaccines are available most often as live vaccines. The use of live vaccines can result in vaccination reactions especially if the birds are stressed (Cserep, 2008).

With the ever expanding flock sizes and the increasing farm size it is possible for individual bird inoculation, which ensures even distribution of the vaccine but involves the chasing and catching of the birds for hours, to constitute a major stress. It also increases labour and cost. To save labour, and possibly minimize stress due to chasing and catching of the birds, it may be possible to administer the two vaccines simultaneously. However, this procedure may have some effects on immune responses to the vaccines, feed consumption and weight gain (Okwor et al., 2013). Although the diseases are the major health constraints responsible for marked economic losses in a country, the dynamicity and the status of the disease in chickens in the study areas have not been yet studied to a full extent and not well documented.

MATERIALS AND METHODS

Study setting

Cross-sectional study type was conducted from October 2015 to April 2016 in selected districts of North Gondar zone, Amhara National Regional State, located in the northwestern part of Ethiopia. The study districts included were Dabark, Dembiya, and Metema, located between 700 and 778kms northwest of the capital, Addis Ababa. The study zone is located between geographically coordinates 12.3° to 13.38° north latitudes and 35.5° to 38.3° east longitudes and the altitude ranges from 550 to 4620 meters above sea level (masl) in western lowland and in north Semen Mountain, respectively. The average annual rain fall vary from 880mm to 1772 mm, which is characterized by a monomodal type of distribution. The mean annual minimum and maximum temperature is 10°C in the highland and 44.5°C in the lowland (Eshete, 2002).

The indigenous chicken population in the study zone is estimated at 3.75million. Most of the poultry is found in the highland and mid highland areas of the region, which is associated with the ecology and human demography. Indigenous chickens are major family poultry types in the area managed under backyard free-range system (Fentie et al., 2013).

Sampling procedure and study population

In this study, a cross-sectional study design was used and applied to collect 384 serum samples from chickens which were selected by simple random sampling method (lottery drawing methods). Serum sample were collected from three different districts which were Dembiya, Dabark and Metema. The serum sample collections were also considered different parameters like sex, age, breed, infection, and vaccination during the data collection time. The serum sample was collected from October 2015 to April 2016. The laboratory tests were conducted in Deberzit National Veterinary Institute, Ethiopia from April to May 2016 using the laboratory techniques of Haemagglutination inhibition test and Indirect-ELISA test for NCD and IBD respectively. The sample size was determined using the formula described by Thrusifield (1995). The study was considered an expected prevalence of 50% and an absolute precision of 5% (5% significance level) with 95% CI. For both IBD and NCD, the total serum sample in Metema, Dembiya and Dabark were collected 114, 92 and 178 respectively.

Data collection instrument and procedures

Totals of 384 chicken serums were collected both from local and exotic (Faso T44) breeds of chickens in the three districts and 2-3 ml of blood were collected from the humeral region of the wing vein with a syringe and needle of 5ml size. The syringe with blood, then kept horizontally until the blood clots. After clotting, the syringe was returned to a vertical, but invert position and left on a bench overnight to permit the serum to ooze out. The separated serum was transferred into Eppendorf tubes, labeled and stored at -20 Cuntil. The Haemagglutination-inhibition test (HIT) and indirect ELISA tests were carried out. HIT and Indirect ELISA tests were performed to detect antibodies against NCD and IBD respectively.

To cite this paper: Birhan Mas., Birhan Mal., Tesfaye Sh, and Tariku A (2019). Detection of antibodies against Newcastle and infectious bursal disease on chicken in north Gondar zone, Ethiopia. *Online J. Anim. Feed Res*., 9(2): 51-58. www.ojafr.ir

Protocol HIT to detect specific antibodies

i) 0.025 ml of PBS is dispensed into each well of a plastic V-bottomed microtitre plate, ii) 0.025ml of serum is placed into the first well of the plate ,iii) Two fold dilutions of 0.025 ml volumes of the serum are made an exotic the plate, iv) 4 HAU virus/antigen in 0.025 ml is add to each well and the plate is left for a minimum of 30 minutes at room temperature, i.e. about20°C, or 60 minutes at 4°C, v) 0.025 ml of 1% (v/v) chicken RBCs is added to each well and, after gentle mixing, the RBCs are allowed to settle for about 40 minutes at room temperature, i.e. about 20°C, or for about 60 minutes at 4°C if ambient temperatures are high, when control RBCs should be settled to a distinct button, vi) The HI titter is the highest dilution of serum causing complete inhibition of 4 HAU of antigen (Zeleke et al., 2005).

The agglutination is assessed by tilting the plates. Only those wells in which the RBCs stream at the same rate as the control wells (positive serum, virus/antigen and PBS controls) should be considered to show inhibition, vii) The validity of results shall be assessed against a negative control serum, which should not give a titter >1/4 (>22or >log22 when expressed as the reciprocal), and a positive control serum for which the titter should be within one dilution of the known titter. The value of serology in diagnosis is clearly related to the expected immune status of the affected poultry. HI titers may be regarded as being positive if there is inhibition at a serum dilution of 1/16 (24or log24 when expressed as the reciprocal) or more against 4 HAU of antigen. Some laboratories prefer to use 8 HAU in HI tests. While this is permissible, it affects the interpretation of results so that a positive titer is 1/8 (23 or log2 3) or more. Back titration of antigen should be included in all tests to verify the number of HAU used. In vaccinate flocks that are being monitore serologically, it may be possible to identify anamnestic responses as the result of a challenge infection with field virus (Alexander & Allan, 1974), but great care should be exercised as variations may occur from other causes. For example, it has been demonstrated that APMV-3 virus infections of ND-virus-vaccinated turkeys will result in substantially increased titers to NCD (Vui, Lohr et al., 2002).

Protocol Indirect ELISA to detect specific antibodies

Coat plate with antigen using a multichannel pipet and tips; dispense 50 µl antigen solutions into each well of micro titer plate. Tap or shake the plate to ensure that the antigen solution is evenly distributed over the bottom of each well. 2. Wrap coated plates in plastic wrap to seal and incubate overnight at room temperature or 2 hr at 37°C. 3. Rinse coated plate over a sink by filling wells with deionized or distilled water Flick the water into the sink and rinse with water two more times. Block residual binding capacity of plate. 4. Fill each well with blocking buffer dispensed as a stream from a squirt bottle and incubate 30 min at room temperature. 5. Rinse plate three times in water as in step 5 Add Ab to plate. 6. Add 50 µl Ab samples diluted in blocking buffer to each of the coated wells, wrap plate in plastic wrap, and incubate ≥2 hr at room temperature. 8. Fill each well with blocking buffer, vortex, and incubate 10 min at room temperature9. Rinse three times in water as in step 5 Wash the plate7. Rinse plates three times in water as in step 5 and 8. Fill each well with blocking buffer, vortex, and incubate 10 min at room temperature9. Rinse three times in water as in step 5 Wash the plate 7. Rinse plate three times in water as in step 5 Add developing reagent to plate. 10. Add 50 µl developing reagent in blocking buffer to each well, wrap in plastic wrap, and incubate ≥2 hr at room temperature.11. Wash plates as in steps 9 to 11 Add substrate and measure hydrolysis. 12. Add 75 µl MUP or NPP substrate solution to each well and incubate 1 hr at room temperature.13. Monitor hydrolysis qualitatively by visual inspection or quantitatively with a microtitre plate reader. Hydrolysis can be stopped by adding 25 µl of 0.5 M NaOH. Read out visually, hydrolysis of NPP can be detected by the appearance of a yellow color. If using a micro titer plate reader to measure NPP hydrolysis, use a 405-nm filter (Williams et al., 1997).

Washing of RBCs: A total of 5 ml of chicken blood was collected aseptically in a disposable syringe containing 1 mL of sodium citrate (4% solution) as an anticoagulant. The blood was centrifuged at 1500 rpm for 15 min. The plasma and Buffy coat was pipetted off. After washing thrice with phosphate buffer saline (PBS), 1% suspension in PBS was made to be used in HI test (Miers et al., 1983).

Data analysis

Data were managed using both in hard and soft copies. Data entry was done using Microsoft office excels and processed using SPSS version 21 statistical software. Descriptive statistics, Chi-Square and ANOVA tests were computed for all the parameters and to analyze the differences in the Sero-prevalence between sexes, ages, vaccine, infection, breeds and among districts. P< 0.05 was considered to best statistical significant variation.

RESULTS

This study is aimed to make a comprehensive study on the detection of Ab against IBD and NCD of Chicken by considering multi factors analysis namely: age, district, vaccination, infection, breed, and Sex. As it is stated in the methodology section, the study considered 384 serum samples in all the factors.

To cite this paper: Birhan Mas., Birhan Mal., Tesfaye Sh, and Tariku A (2019). Detection of antibodies against Newcastle and infectious bursal disease on chicken in north Gondar zone, Ethiopia. *Online J. Anim. Feed Res*., 9(2): 51-58. www.ojafr.ir

Ab Detection of IBD

The results revealed that out of the total 156 male chickens Sero-positive were 81.6%, whereas detection of Ab of females from the total 228 serum samples was found to be 69.4%. The highest Ab detection was found in males (81.6%) as compared to females (69.4%). The findings of this study indicating that 0% Ab in local and 83.3% in exotics do not agree with the findings of (Halima et al., 2007). 314 young chickens a total of 285 serum samples were found Ab positive (90.9%). Likewise, out of the 70 adult chickens a total 0 serum samples were found Ab positive (0.0%).

The results showed that out of the total 48 infected chickens Ab detection were 80%, whereas out of the total 336 non-infected Ab detection 73.6% were found to be Ab positive. The highest Ab detection was found on vaccinated Chickens 83.3% were serum antibody, as compared to non-vaccinated Chickens with 0% Ab detection which is in line with the findings of (Jenbreie et al., 2012).

This indicates there are Sero-positive rate of 72.2%, 89.7% and 67.9% in Metema, Dembiya and Debark district respectively. It shows that compared to the others, Dembiya has highest Ab detection rate among the three districts. These findings agree with the findings of Halima et al. (2007) and Tadesse et al. (2005).The possible explanation for this could be there are few chicken farms in the highland area of the study sites and chicken population number is a factor for the transmission of the disease.

The two-sided *asymptotic significance* of the chi-square statistic (χ^2 = 135.76) was less than 0.05 (P=0.00). Therefore it is safe to say that the differences between vaccinated and non-vaccinated chickens with respect to IBD was not due to chance variation, which implies that vaccinated chickens have a higher level of prevalence on IBD than non-vaccinated chickens. Except sex and disease all the parameters such as district, age, breed shows similar result. However, the findings shows that IBD was independent of sex and infection with P= 0.09 and 0.48 respectively.

Detection of Antibody to Infection Bursal Disease Virus by Sex, Age and Breed

In this section, detection serological antibodies of IBDV of chicken are investigated by sex, age and breed. As it is shown in Table 2, the findings of sex, age and breed are listed.

Table 1 - Detection of antibodies against infectious bursal disease by sex, age and breed

Variable	No examined Chickens	No. antibody Positive	Antibody detection	Total antibody detection	χ2(P-value)	P-value*
Male	156	127	81.6%	285(74.4%)	71.1(0.09)	0.07
Female	228	158	69.9%			
Adult	70	0	0.0%	285(74.4%)	246.44(0.00)	0.00
Young	314	285	90.0%			
Local	42	0	0.0%	285(74.4%)	135.16(0.00)	0.00
Exotics	342	342	83.3%			

χ2= the chi-square value, *=the annova p-value

Table 2 - Detection of antibodies against infectious bursal disease by vaccinated, infection and districts

Variable	No. Examined Chickens	No. antibody positive	Antibody Detection Rate	Total Antibody rate	χ2 (P-value)	P-value
Vaccinated	342	285	83.3%	285(74.4%)	135.76(0.00)	0.00
Non-vaccinated	42	0	0.0%			
Infected	48	38	80.0%	285(74.4%)	0.7(0.48)	0.345
Non- infected	336	247	73.6%			
Dembiya	92	82	89.7%	285(74.4%)	14.63(0.01)	0.00
Dabark	114	82	72.2%			
Metema	178	120	67.9%			

Ab Detection of NCD

The 156 male chickens Serological Ab positive were account 79.57% and the 228 female Chickens also account 79.56%. This shows that both male and female Chickens have equal serum Ab with respect to NCD, which indicates being male or female is not a decisive factor in the resistance of NCD. And from the total 32 local chickens Ab positive were 76.19%, whereas, exotics or Faso T44 chickens from the total 274 serum samples were found to be

To cite this paper: Birhan Mas., Birhan Mal., Tesfaye Sh, and Tariku A (2019). Detection of antibodies against Newcastle and infectious bursal disease on chicken in north Gondar zone, Ethiopia. *Online J. Anim. Feed Res.*, 9(2): 51-58. www.ojafr.ir

80.12% Ab positive. The highest Ab detection was found in exotics (8.12%) as compared to local (76.19%) I agree with (Chaka et al., 2013).

In addition to this, further comparisons between local and exotics (Faso T44) Chickens were made. The result of the findings is similar to Moges (2010) reports. It shows that there no statistical significance difference (P-Value =0.113) between local and exotics or Faso T44 Chickens with respect to NCD. It shows that, out of the total of 286 young chickens Sero-positive were 91.1%, whereas the serological prevalence of adult from the total 70 serum samples was found to be 28.6%. The highest prevalence was found in young (91.1%) as compared to adult (28.6%). The results of the study indicating 91.1% Ab in young and 28.6% in adult agree with those of who reported highest prevalence in young and the lowest in the adult.

The results revealed that the Serological Ab of Metema was found to be 86.0%, that of Dembiya was 92.4% and that of Dabark was 69.1 %. Among them, the highest Ab detection was found in Dembiya (92.4%) as compared to Metema (86.0%) and Dabark (69.1%). The results of the study indicating 86.0% Ab in Metema, 92.4% in Dembiya chickens and 69.1% in Dabark was however, observed that susceptibility was the highest to Debark as compared to Metema and Dembiya which was in line with previous studies I agree with (Ashenafi & Eshetu, 2004).

The results revealed that out of the total 342 vaccinated and 42 non-vaccinated chickens 89.5% and 0%, serological Ab detection is respectively found. When it was represented proportionally the total of 38 infected chickens Serological Ab were account 79.2%, whereas the serological Ab of non-infected from the total of 286 serum samples was found to be 79.8%, which shows that there is no serological Ab difference between infected and non-infected Chickens with respect to NCD.

The two-sided *asymptotic significance* of the chi-square statistic (χ^2 = 135.76) is less than 0.05 (P= 0.00). Therefore it is safe to say that the differences between vaccinated and non-vaccinated chickens with respect to NCD is not due to chance variation, which implies that vaccinated chickens have higher level of prevalence on NCD than non-vaccinated chickens. Similarly the report in the same table shows that except sex and infection all the parameters such as district, age, breed show similar result. However, the finding show that NCD was independent of sex and breed with P= 0.036 and 0.551 respectively.

Table 3 - Detection of antibodies against Newcastle disease by sex, age and breed

Variable	No. examined chickens	No. Positive	Antibody Detection rate	Total Antibody detection rate	χ2(p-value)	p-value
Male	156	124	79.59%	306(79.9%)	0.007(0.936)	0.999
Female	228	182	79.56%			
Adult	70	20	28.6%	306(79.9%)	138.19(0.00)	0.0001
Young	314	286	91.19%			
Local	42	32	76.19%	306(79.9%)	0.36(0.551)	0.0001
Exotics	342	274	80.12%			

Table 4 - Detection of antibodies against Newcastle disease by vaccine, infection and districts.

Variable	No. examined chickens	NO. antibody positive	Antibody detection Rate	Total antibody Rate	χ2(p-value)	p-value
Vaccinated	342	306	89.5%	306 (79.6)	18.1 (0.00)	0.0001
Non-vaccinated	42	0	0.00%			
Infected	42	38	79.2%	306 (79.6)	73.73 (0.00)	0.834
Non- infected	48	268	79.8%			
Dembiya	92	85	92.4%	306 (79.6)	24.27 (0.00)	0.0001
Metema	114	98	86.6%			
Dabark	178	123	69.0%			

DISCUSSION

In vaccinated flocks, positive serological results are clear evidence that the birds have been exposed to the infectious agent under investigation, although without identifying the infecting strains. In the present study, we confirmed from the sellers during purchase that all of them had vaccinated their chickens for any poultry diseases especially cross breeds. Hence, the presence of antibodies to NCD and IBD was considered evidence of not the exposure to natural infection.

The study revealed that the prevalence of NCD antibodies in backyard chickens was generally high, around 76.19%. This is considerably higher than previous reports by Geresu et al. (2016) who reported prevalences of 19.8%

To cite this paper: Birhan Mas., Birhan Mal., Tesfaye Sh, and Tariku A (2019). Detection of antibodies against Newcastle and infectious bursal disease on chicken in north Gondar zone, Ethiopia. *Online J. Anim. Feed Res.*, 9(2): 51-58. www.ojafr.ir

in the southern and Rift Valley districts and 32.2% in central Ethiopia, respectively, but our results were not close to those reported by (Regasa, Fufa, Berihanu, & Hunduma, 2007) in southern Ethiopia (11%). Our results are also consistent with seroprevalence in backyard poultry of 76.19% in Mauritania. When chickens are affected by a velogenic NCD virus that results in very high mortality, one is likely to find few or no survivors with antibodies. Up to 30% of market sellers claimed to have observed poultry disease signs (sudden death, diarrhea, and nervous signs) resembling Newcastle disease during previous months. There was no observed seasonal or geographic variation in seroprevalence, in the present study, suggesting that the disease is widespread and occurs throughout the year in the study area.

The survey also indicated that IBD is widespread among village chickens in the study area, with a seroprevalence of 74.4%. This agrees closely with reports by Degefu et al. (2010) from Ethiopia, and Chaka et al. (2012) from India, who reported seroprevalence of 76.6%, 100, 74, and 73.7% respectively. However, relatively lower IBD seroprevalence were recorded in Mauritania (15.8%; Chrysostome et al., 1995), Zimbabwe (55%; Mazengia et al., 2010). The higher seroprevalence of the disease in the study area, in the apparent absence of mortality, could be due to an IBD virus of lower pathogenicity, unlike the case reported from the Amhara region of Ethiopia (Mazengia et al., 2009) or the outbreak in a commercial broiler farm with evident mortalities (Jenberie et al., 2014). It is also possible that the birds were infected with IBD virus as adults, at which stage they simply seroconvert without any apparent clinical disease. With such a high seroprevalence and low mortality of infected birds, there is the possibility of genetic resistance among indigenous breeds of chickens in Ethiopia, as reported from Egypt (Hassan, 2004). This is difficult to demonstrate using serological studies, but further studies could be undertaken to investigate this.

CONCLUSION

In conclusion, these research findings extend the presence of detectable antibodies to NCD and IBD in chickens, in the three districts of North Gondar Zone. As it is known Chickens' which might found in different districts have been high variable detectable antibodies if they are either susceptible to the pathogenic or vaccinated and either female or male.

DECLARATIONS

Acknowledgment

First of all, the authors would like to express their sincere gratitude to the study participants for their willingness to take part in the study. The authors' heartfelt thanks will also go to the University of Gondar, Vice President of research and community service office for the financially supporting the study.

Authors' information

Corresponding Author: maste675@gmail.com (Mastewal Birhan). Mastewal Birhan (MB1): maste675@gmail.com; Malede Birhan (MB2): birhan1975@gmail.com; Shimelis Tesfaye (ST): shimelsrich@gmail.com; Amare Tariku (AT): amaretariku15@yahoo.com;

Authors' contributions

MB1 conceived the study, coordinated the overall activity, and carried out the statistical analysis, drafted the manuscript. ST participated in drafting and reviewing the manuscript. MB2 conceived the study, coordinated the overall activity, and reviewed the manuscript. ST participated in drafting and reviewing the manuscript. AT participated in the design of the study, and reviewed the manuscript. All authors read and approved the final manuscript.

Availability of data and materials

Data will be made available upon request of the primary author

Consent to publish

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Funding

This study was funded by the University of Gondar. The views presented in the article are of the authors and do not necessarily express the views of the funding organization. The University of Gondar was not involved in the design of the study, data collection, analysis, and interpretation.

REFERENCE

Ali, AS, Abdalla, MO, & Mohammed, MEH. (2004). Interaction between Newcastle disease and infectious bursal disease vaccines commonly used in Sudan. *Int. J. Poult. Sci, 3*(4), 300-304.

Anebo, Zelalem Gebretsadik, Teklemichael, Kidist, Bacha, Belachew, Habte, Tadios, & Hunde, Addisalem. (2014). Evaluation of the newcastle disease antibody level after vaccination regimes in chickens in Debrezeit Agricultural Research Center, Ethiopia. *Journal of Veterinary Medicine and Animal Health, 6*(1), 7-12.

Ashenafi, H, & Eshetu, Y. (2004). Study on gastrointestinal helminths of local chickens in central Ethiopia. *Revue de médecine vétérinaire, 155*, 504-507.

Awan, Manzoor Ahmed, Otte, MJ, & James, AD. (1994). The epidemiology of Newcastle disease in rural poultry: a review. *Avian pathology, 23*(3), 405-423.

Chaka, Hassen, Goutard, Flavie, Bisschop, Shahn PR, & Thompson, Peter N. (2012). Seroprevalence of Newcastle disease and other infectious diseases in backyard chickens at markets in Eastern Shewa zone, Ethiopia. *Poultry Science, 91*(4), 862-869.

Chaka, Hassen, Goutard, Flavie, Gil, Patricia, Abolnik, Celia, de Almeida, Renata Servan, Bisschop, Shahn, & Thompson, Peter N. (2013). Serological and molecular investigation of Newcastle disease in household chicken flocks and associated markets in Eastern Shewa zone, Ethiopia. *Tropical animal health and production, 45*(3), 705-714.

Chrysostome, CAAM, Bell, JG, Demey, F, & Verhulst, A. (1995). Sero prevalences to three diseases in village chickens in Benin. *Preventive veterinary medicine, 22*(4), 257-261.

Cserep, Tibor. (2008). Vaccines and vaccination. *Poultry diseases*, 66-81.

Degefu, Hailu, Balcha, M, Yohannes, M, & Getachew, M. (2010). Seroprevalence of infectious bursal disease in backyard chickens of Oromia regional state, Ethiopia. *Veterinary Research, 3*(4), 89-93.

Eshete, Abeje. (2002). Regeneration status, soil seed bank and socio-economic importance of Boswellia papyrifera (Del.) Hoschst. In two woredas of North Gondar zone, Northern Ethiopia.

Ezema, WS, Okoye, JOA, & Nwanta, JA. (2009). LaSota vaccination may not protect against the lesions of velogenic Newcastle disease in chickens. *Tropical animal health and production, 41*(4), 477-484.

Fentie, Tsegaw, Abebe, Birhanu, & Kassa, Tesfu. (2013). Small-scale family poultry production in north Gondar: characteristics, productivity and constraints. *Livestock Research for Rural Development, 25*, 161.

Geresu, Minda Asfaw, Elemo, Kemal Kedir, & Kassa, Gezahegne Mamo. (2016). Newcastle disease: Seroprevalence and associated risk factors in backyard and small scale chicken producer farms in Agarfa and Sinana Districts of Bale Zone, Ethiopia. *Journal of Veterinary Medicine and Animal Health, 8*(8), 99-106.

Halima, HFWC, Neser, FWC, Van Marle-Koster, E, & De Kock, A. (2007). Village-based indigenous chicken production system in north-west Ethiopia. *Tropical animal health and production, 39*(3), 189-197.

Hassan, MK. (2004). Very virulent infectious bursal disease virus in Egypt: epidemiology, isolation and immunogenicity of classic vaccine. *Veterinary research communications, 28*(4), 347-356.

Jenberie, Shiferaw, Lynch, Stacey E, Kebede, Fekadu, Christley, Robert M, Gelaye, Esayas, Negussie, Haileleul, . . . Ayelet, Gelagay. (2014). Genetic characterisation of infectious bursal disease virus isolates in Ethiopia. *Acta tropica, 130*, 39-43.

Jenbreie, Shiferaw, Ayelet, Gelagay, Gelaye, Esayas, Kebede, Fekadu, Lynch, Stacey E, & Negussie, Haileleul. (2012). Infectious bursal disease: seroprevalence and associated risk factors in major poultry rearing areas of Ethiopia. *Tropical animal health and production, 45*(1), 75-79.

Mazengia, Hailu, Bekele, ST, & Negash, Tamiru. (2009). Incidence of infectious bursal disease in village chickens in two districts of Amhara Region, Northwest Ethiopia. *Livestock Research for Rural Development, 21*, 12.

Mazengia, Hailu, Tilahun, SB, & Negash, T. (2010). Newcastle Disease and Infectious Bursal Diseases are Threat to Village Chicken Production in Two Districts of Amhara National Regional State, Northwest Ethiopia. *IUP Journal of Life Sciences, 4*(2).

Miers, LA, Bankowski, RA, & Zee, YC. (1983). Optimizing the enzyme-linked immunosorbent assay for evaluating immunity of chickens to Newcastle disease. *Avian Diseases*, 1112-1125.

Moges, Fisseha. (2010). *Indigenous chicken production and marketing systems in Ethiopia: Characteristics and opportunities for market-oriented development*: ILRI (aka ILCA and ILRAD).

Negash, Tamiru, Gelaye, Esayas, Petersen, Henning, Grummer, Beatrice, & Rautenschlein, Silke. (2012). Molecular evidence of very virulent infectious bursal disease viruses in chickens in Ethiopia. *Avian diseases, 56*(3), 605-610.

Okwor, EC, Eze, DC, Anyanwu, MU, Okpe, CB, & Eze, PC. (2013). Effects of mixed vaccinations against Newcastle disease and infectious bursal disease on immune response feed consumption and weight gain in broilers. *Journal of Agriculture and Veterinary Science, 6*(3), 63-68.

Okwor, Emmanuel Chukwudi, Eze, Didacus Chukwuemeka, & Okonkwo, Kodi. (2012). Serum antibody levels against infectious bursal disease virus in Nigerian village chickens. *Pak Vet J, 32*(2), 286-287.

Regasa, C, Fufa, D, Berihanu, S, & Hunduma, D. (2007). *Seroprevalence of Newcastle disease in backyard chickens in mid Rift Valley of Oromia, Ethiopia.* Paper presented at the 12th International conference of the Association of Institutions of Tropical Veterinary Medicine, Montpellier.

Sambo, Emmanuel, Bettridge, Judy, Dessie, Tadelle, Amare, Alemayehu, Habte, Tadiose, Wigley, Paul, & Christley, Robert M. (2015). Participatory evaluation of chicken health and production constraints in Ethiopia. *Preventive veterinary medicine, 118*(1), 117-127.

Tadesse, Serkalem, Ashenafi, Hagos, & Aschalew, Zeleke. (2005). Seroprevalence study of Newcastle disease in local chickens in central Ethiopia. *International Journal of Applied Research in Veterinary Medicine, 3*(1).

Vui, Tran Quang, Lohr, JE, Kyule, MN, Zessin, KH, & Baumann, MPO. (2002). Antibody levels against Newcastle disease virus, infectious bursal disease virus and avian influenza virus in rural chickens in Viet Nam. *Int. J. Poult. Sci, 1*(5), 127-132.

To cite this paper: Birhan Mas., Birhan Mal., Tesfaye Sh, and Tariku A (2019). Detection of antibodies against Newcastle and infectious bursal disease on chicken in north Gondar zone, Ethiopia. *Online J. Anim. Feed Res*., 9(2): 51-58. www.ojafr.ir

Williams, Roy, Boshoff, Chris H, Verwoerd, Dirk, Schoeman, Mara, van Wyk, Antonette, Gerdes, Truuske H, & Roos, Katy. (1997). Detection of antibodies to Newcastle disease virus in ostriches (Struthio camelus) by an indirect ELISA. *Avian diseases*, 864-869.

Wilson, RT. (2010). Poultry production and performance in the Federal Democratic Republic of Ethiopia. *World's Poultry Science Journal,* 66(3), 441-454.

Yongolo, MGS, Machangu, A Maeda, & Minga, UM. (2002). Newcastle disease and Infectious bursal disease among free-range village chickens in Tanzania. *Characteristics and parameters of family poultry production in Africa. IAEA, Vienna*.

Zeleke, Aschalew, Gelaye, Esayas, Sori, Teshale, Ayelet, Gelagay, Sirak, Asegedech, & Zekarias, Bereket. (2005). Investigation on infectious bursal disease outbreak in Debre Zeit, Ethiopia. *International Journal of poultry science, 4*(7), 504-506.

To cite this paper: Birhan Mas., Birhan Mal., Tesfaye Sh, and Tariku A (2019). Detection of antibodies against Newcastle and infectious bursal disease on chicken in north Gondar zone, Ethiopia. *Online J. Anim. Feed Res*., 9(2): 51-58. www.ojafr.ir

2019 SCIENCELINE

Online Journal of Animal and Feed Research

Volume 9, Issue 2: 59-67; March 25, 2019 ISSN 2228-7701

EFFECTS OF LOW TEMPERATURE UPON HATCHABILITY AND CHICK QUALITY OF ROSS-308 BROILER BREEDER EGGS DURING TRANSPORTATION

Ahmad HUSSAIN[1], Muhammad BILAL[2], Faiza HABIB[2], Barkat Ali GOLA[2], Peer MUHAMMAD[2], Amanullah KAKER[2], Adnan YOUSAF [3]✉, Rabia KHALIL[3]

[1]*Department of Poultry Husbandry, Sindh Agriculture University Tandojam, Pakistan*
[2]*Department of Livestock and Dairy Development Quetta, Baluchistan, Pakistan*
[3]*Salman Poultry (Pvt) Limited, Rawalpindi, Pakistan*

✎*Supporting Information*

ORIGINAL ARTICLE
PII: S222877011900009-9
Received: January 09, 2019
Revised: March 15, 2019

ABSTRACT: **In current experiment n=20,000 Ross-308 broiler breeder eggs collected from 33 week old flocks were subjected to cold stress during transportation. Eggs were allocated to 4 temperature groups (treatments): 1.5°C, 2°C, 3.5°C, 5°C, and a control group, 20°C. Each treatment had 4,000 eggs, and data were analyzed in a completely randomized design. The results of this study showed that cold stress had a significant effect on percentage of egg weight loss (P<0.001), and minimal egg weight loss occurred in the control group. The percentage of exploders and early hatched chicks and chick weight were higher in the below 3°C temperature treatment than the other groups (P<0.01). Cold stress had a significant effect on chick length, hatchability, and the hatching of fertile eggs (P<0.001). The effects of cold stress on chick yield and body weight uniformity were significant (P<0.01). The effect of cold stress on hatchery by product efficiency was significant (P<0.001), but did not affect fertility. Cold stress also had significant effects on early (1-8 days), middle (9-17 days), and late mortality (20-21 days); total embryo mortality; and exposed brain. Ectopic viscera was significant (P<0.001), and most mortality was observed in below 4°C treatments. Total percentages of malpositions and deformity (P<0.001) and egg contamination at 1-9 days (first stage) and 10-21 days (second phase) were affected by cold stress (P<0.001). Cold stress also had a significant impact on the number of cull chicks; percent of string navel, button navel, total string, and button; omphalitis; full body cavity; red hocks; dehydration; dirty chickens; and stubby down. Cold stress affects performance during incubation and overall chick quality.**

Keywords: **Cold Stress, Egg Transportation, Hatchability, Chick Quality, Malposition And Deformity, Culls, Egg Weight Loss, Embryo Mortality And Contamination**

INTRODUCTION

Incubation provides proper conditions for fertile eggs to produce high quality day old chicks. This occurs when the fertile eggs are delivered under standard conditions from breeder farm to hatchery (Jabbar and Yousaf 2017, Nakage et al., 2003). It is necessary to protect hatching eggs against any heat or cold stress during collection in the barn; storage at the farm; and transportation to the hatchery, egg grading room, incubator, and hatcher. For example, Cobb-Vantress, is one of the primary breeders recommends that the temperature of trucks delivering hatching eggs from farm to hatchery be between 20-23°C (Yousaf et al., 2017, Cobb-Vantress, 2014). Temperature is one of the important factors affecting the growth and development of the embryo at all stages of the incubation period (Yousaf et al., 2016). The optimum temperature for fertile eggs during the storage period depends on the age of eggs, the age of broiler breeder flock, and the genetic strain of the birds (Hussain et al., 2018, Tullett et al., 2009). It must be noted that, as storage time increases, hatching eggs should be stored at lower temperatures (Yousaf et al., 2017). Guidelines are provided by primary breeder companies. For instance, Aviagen recommends storing eggs 1-3 days or older (e.g., 4 days) at 19 °C and 16-18°C, respectively (ROSS, 2016). It has been shown that storing eggs at 18.33°C for 10 h reduced the internal temperature of the eggs to 1 °C (Yousaf et al., 2017).

However, this did not have any impact on hatchability rate (Taylor et al., 2003). Storing turkey hatching eggs at 18.33 °C for 1, 2, 3, and 4 h led to a higher number of female embryos compared to eggs stored from 10 to 12.7°C; when the temperature was reduced to 16°C, hatchability decreased (Jull et al., 2001). If the temperature of the egg center over 120 min reaches 7 to 10°C, embryos will develop normally until they reach 8 days of age (Taylor et al., 2006). Keeping eggs at 3°C for 4 days resulted in the development of the embryos to the blood ring stage with a

To cite this paper: Hussain A, Bilal M, Habib F, Gola BA, Muhammad P, Kaker A, Yousaf A, Khalil R (2019). Effects of low temperature upon hatchability and chick quality of Ross-308 broiler breeder eggs during transportation. *Online J. Anim. Feed Res.*, 9(2): 59-67. www.ojafr.ir

wide and enlarged blastoderm after which only the ectoderm and endoderm had the opportunity to grow (Taylor et al., 2006). Cold stress 2°C before incubation increased embryo mortality, and the peak of mortality occurred after 16 days (Wilson, 2001). Eggs maintained at 2 to 3°C caused ice crystal formation, which led to irreversible damage to embryo tissues (Hussain et al., 2018). It was reported that cold stress reduced yolk consumption by the embryo. It also prevented growth of the embryo and reduced the water loss or vapor exchange of the egg surface (Yousaf et al., 2017). A reduction of ambient temperature in broiler breeder hens increased food consumption and serum corticosterone (Yousaf, 2016). It reduced egg production; feed efficiency; and concentrations of some vitamins, minerals, insulin, ascorbic acid, alpha tocopherol, and retinol in plasma (Yousaf, 2016). However, the level of malondialdehyde; the production rate of free radicals; and requirements for vitamins A, C, and E increased (Ensminger et al., 2004, Siegel et al., 2003, Klasing et al., 2003, Kucuk et al., 2003). Cold stress caused weakness in lymphoid organs and decreased immune function in hens. The effects of cold stress in chickens can be produced by different mechanisms (Yousaf, 2016). The cold changes the endocrine system; the hypothalamus-pituitary-adrenal axis, sympathetic-adrenal-medullary axis, and the thypothalamus-pituitary-thyroid axis become active, and as a result, hormone responses to stress will be evident (Hussain et al., 2018). Cold stress caused a significant drop in corticosterone levels and affected cellular immunity, but had no effect on T4 (Hangalapura et al., 2004). Several climate changes may take place during egg transportation. From an economic perspective it is a very important issue for day old chick producers (Jabbar and Yousaf, 2017). Therefore, in the current study the effects of cold stress and freezing conditions on broiler breeder eggs during transportation, and subsequent chick quality and incubation characteristics were investigated.

MATERIALS AND METHODS

Ethical Approval

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

Selection of Site

The experiment started in January at menshera-Pakistan, when the ambient temperature during delivery of the hatching eggs to the hatchery was about -4 to 0°C. Due to road closures as a result of cold weather conditions, all eggs were kept inside the truck for 40 h.

Analyzing eggs internal Temperature

Internal temperature of the eggs was measured by thermometer (in 42 eggs, by breaking the eggs and measuring the temperature at the large end). The thermometer (Testo, Co.) was capable of measuring temperatures from -40 to 230°C, with accuracy to 0.1°C. Temperature recording for each egg took about 90 s. We did our best to minimize the effect of ambient temperature on the thermometer. A total of 20,000 fertile and settable eggs were used in this study. Of this total number, 12% (equal to 7 cartons) were selected; 6 eggs from each carton. In total, the internal temperatures of 42 eggs (7× 6) were measured.

Classification of groups

There were a total of 4 treatments (temperature group) 1.5°C, 2°C, 3.5°C, 5°C, and a control group, 20°C. Each treatment had 4 replicates, and each replicate contained 1000 eggs.

Pre Warming Regimen

In a completely randomized design, eggs were prewarming for 8 h at 82°F. Egg prewarming was completed in 8 h which is 3 h more than the usual time allotted.

Egg weight sample

From each treatment 165 eggs (825 eggs total) were individually weighed by digital scale (Berlini, model KV 2001). Eggs were individually weighed while in the setter and at the time of transfer from setter trays to the hatcher, at day 19 of incubation (449 h). In the incubator, eggs usually lose part of their weight as water vapor (egg weight loss) (Tullett et al., 2009). To calculate the percentage of egg weight loss, the following formula was used:

$$\text{Weight of eggs} = \frac{\text{weight before Pre warming} - \text{weight before transfer} \times 100}{\text{Weight before Pre warming}}$$

It must be noted that we were calculated pre-warming weight and not setting weight, as there might have been some weight loss during the 5 to 8 h of pre-warming.

To cite this paper: Hussain A, Bilal M, Habib F, Gola BA, Muhammad P, Kaker A, Yousaf A, Khalil R (2019). Effects of low temperature upon hatchability and chick quality of Ross-308 broiler breeder eggs during transportation. *Online J. Anim. Feed Res.*, 9(2): 59-67. www.ojafr.ir

Incubation Regimen

After completion of pre-heating stage the incubator started automatically stage profile (Ross prime age recommended by Chicks Master, USA). In order to synchronize and prevent delay in the removal of chicks from the hatcher, the egg setting was changed to 1 h earlier than the usual time. Every effort was made to prevent eggs from sweating while they were still in the preheating room.

Candling Process

After completing 456 h in incubator the fertility of eggs was checked through candling process. All the infertile eggs were removed from the tray and just fertile eggs shifted to hatchers for next 50 hours (2 days and 8 hours) (Jabbar and Yousaf, 2017). CobbVanress, Inc. recommends candling between days 10 and 12 of incubation (Cobb-Vantress, 2014); however, the Aviagen management guide indicates that the right time for candling is day 18 of incubation (ROSS, 2016). Measuring chick length is a fast method for evaluating chick quality (Molenaar et al., 2009). We measured from the beginning of the beak to the end of the middle toe by ruler. Chicks were stretched along a ruler by HatchTech method (Molenaar et al., 2009). Only 25 chicks are needed to measure length; however, we evaluated 150 chicks (Molenaar et al., 2009). We calculated the chick yield (%) or chick weight/initial egg weight ratio from this formula (Tullett et al., 2009):

$$\text{Chick weight} = \frac{\text{Chick weight (g)} \times 100}{\text{Initial eggs weight (g)}}$$

DIS Analysis

We also performed an egg breakout on unhatched eggs (equivalent 20.83% of total), and results were recorded and evaluated. After the hatch, all residue, dead embryos, debris, culls, and shells were collected to buried far away from hatchery.

Statistical Analysis

All data were analyzed with SAS software version 9.1. Statistical models used for data analysis were as follows (Zamani, 2010).

RESULTS

The data showed that the rate of egg weight loss during transportation from farm to hatchery was 1.49%. It must be mentioned that the ambient temperature during transport from broiler breeder farm to the hatchery reached below zero. This did not cause freezing in the egg yolks or albumen; however, wrinkling of the vitelline membrane and color spots in egg yolks (manifested as darker or lighter areas) were observed. In this study egg weight loss was affected by temperature ($P<0.001$), and maximum weight loss (14.20%) was found in treatment 2. A number of contaminated eggs exploded during the transfer stage or incubation.

In this study, percentage of exploded eggs was influenced by temperature ($P<0.01$); most exploders (0.05%) were in treatment 1. The number of early hatched chicks in the incubator was significantly higher in treatment 1 ($P<0.001$) than in other treatments. Some chicks pipped the outer shell of the egg while still in the incubator. Under normal conditions we observed 1%-1.5% of eggs in this situation. As indicated in Table 1, cold stress did not have a significant effect on eggs pipped. The effect of cold stress on chick weight was significant ($P<0.01$), and mean weight of chicks in treatment 1 (1.5 °C) was higher than in other treatments. Moreover, chick yield in treatment 1 was higher than in other treatments ($P<0.01$). Chick length was affected by cold stress ($P<0.001$); minimum chick length (17.90 cm) was observed in treatment 1, and the greatest chick length was found in the control group. Chick length decreased with reduction in temperature during transport.

As shown in Table 2, hatchability and hatch of fertile eggs decreased as the severity of cold stress increased. Cold stress had a significant effect on percent hatchability and hatch of fertile eggs ($P<0.001$). Cull percentage and hatchery byproduct efficiency increased when temperature was reduced ($P<0.001$); cold stress also significantly reduced the percentage of chick weight uniformity ($P<0.01$). As presented in Table 3, increase in cold stress before incubation resulted in a significant increase in the rate of contamination ($P < 0.001$). Cold stress also affected total embryo mortality ($P<0.001$), and, with decrease in temperature below 4 °C, total mortality increased. Reducing the temperature did not affect the infertility rate of eggs. Ectopic viscera (ECV) are a condition in which the intestines appear outside the abdominal cavity when the chicken is fully developed (Tullett et al., 2009).

In this study, cold stress significantly affected the incidence of ECV ($P<0.001$); with a decrease in temperature, the rate of ECV increased. As shown in Table 4, most malpositions (1.10%) were observed in treatment 1. It seemed that temperatures below 2 °C increased the incidence of malpositions. Total malpositions and also head between thighs, beak above right wing, and head under left wing increased. Cold stress caused a significant increase in the

To cite this paper: Hussain A, Bilal M, Habib F, Gola BA, Muhammad P, Kaker A, Yousaf A, Khalil R (2019). Effects of low temperature upon hatchability and chick quality of Ross-308 broiler breeder eggs during transportation. *Online J. Anim. Feed Res.*, 9(2): 59-67. www.ojafr.ir

rate of malpositions in treatments compared to the control group (P<0.001). As indicated in Table 5, cold stress caused significant increases in the incidence of chicken deformities in treatments compared to the control group. As shown in Table 6, cold stress significantly increased the number of chicks culled due to conditions such as red hocks, button navel, summation of button, and string navel.

Table 1 - Effect of low temperature on incubation performance and chick quality

Treatment	Initial egg weight (g)	Egg weight loss %	Exploder %	Early hatched %	Eggs Pipped %	Chick weight (g)	Chick length (cm)	Chick Yield %
1	61.76	13.66 ^{a}	0.056 ^{a}	0.016 ^{a}	0.66 ^{ab}	42.83 ^{a}	17.90 ^{c}	69.35 ^{a}
2	61.80	14.20 ^{a}	0.015 ^{b}	0.0066 ^{b}	1.206 ^{a}	41.67 ^{b}	17.98 ^{c}	67.43 ^{bc}
3	61.77	13.52 ^{a}	0.015 ^{b}	0.00 ^{c}	1.14 ^{a}	41.53 ^{b}	18.07 ^{c}	67.23 ^{bc}
4	61.83	14.06 ^{a}	0.023 ^{b}	0.0046 ^{bc}	1.19 ^{a}	41.34 ^{b}	18.30 ^{b}	66.86 ^{c}
Control	61.71	12.16 ^{b}	0.020 ^{b}	0.00 ^{c}	0.02 ^{b}	41.93 ^{b}	18.61 ^{a}	67.95 ^{b}
SEM	0.187	0.222	0.0053	0.002	0.292	0.210	0.079	0.291
P Value	0.992	0.0005	0.0012	0.007	0.0674	0.0042	0.0002	0.0011

$^{a-c}$: Means within a column without a common superscript differ significantly (P≤0.05). Treatments: 1= (1.5°C), 2= (2°C), 3= (3.5°C), 4= (5°C), control= (20°C).

Table 2 - Effect of low temperature on hatchability, culls, uniformity, fertility, hatchery by product efficiency

Treatment	Hatchability %	Hatch of fertile %	Culls %	Uniformity of chicks weight %	Fertility %	Hatchery by products efficiency %
1	85.40^{e}	87.31 ^{d}	1.84 ^{a}	73.63 ^{d}	97.81	51.35 ^{a}
2	86.39 ^{d}	88.28 ^{c}	1.49 ^{b}	77.57 ^{cd}	97.85	51.19 ^{ab}
3	86.85 ^{c}	88.72 ^{c}	1.21 ^{c}	80.36 ^{bc}	97.89	51.02 ^{bc}
4	87.84 ^{b}	89.86 ^{b}	1.09 ^{d}	82.50 ^{ab}	97.75	50.96 ^{c}
Control	90.36 ^{a}	92.58 ^{a}	0.84 ^{c}	85.33 ^{a}	97.60	50.30 ^{c}
SEM	0.095	0.219	0.029	1.400	0.250	0.070
P Value	<0.0001	<0.0001	<0.0001	0.0014	0.9262	<0.0001

$^{a-c}$: Means within a column without a common superscript differ significantly (P≤0.05). Treatments: 1= (1.5°C), 2= (2°C), 3= (3.5°C), 4= (5°C), control= (20°C).

Table 3 - Effect of low temperature on infertility, embryo mortality and contamination

Treatment	Infertility	1-8 days mortality	9-17 days mortality	18-19 days mortality	20-21 days mortality	Total mortality	Contamination		Ectopic viscera
							1-9 days	10-21 days	
1	2.19	6.15^{a}	2.20 ^{a}	1.53 ^{a}	0.88 ^{a}	10.77 ^{a}	1.21 ^{a}	1.21 ^{a}	0.28 ^{a}
2	2.14	5.24 ^{b}	1.78 ^{c}	2.02 ^{ab}	0.85 ^{b}	9.90 ^{b}	1.10 ^{b}	0.73 ^{b}	0.17 ^{b}
3	2.10	5.47 ^{b}	2.01 ^{b}	2.60 ^{a}	0.84 ^{b}	10.94 ^{a}	1.00 ^{c}	0.66 ^{c}	0.13 ^{c}
4	2.25	4.39 ^{c}	1.08 ^{d}	2.59 ^{a}	0.81 ^{c}	8.87 ^{c}	0.58 ^{d}	0.67 ^{c}	0.09 ^{d}
Control	2.40	3.48 ^{d}	0.87 ^{c}	1.86 ^{b}	0.78 ^{d}	7.00 ^{d}	0.24 ^{e}	0.1 ^{d}	0.04 ^{e}
SEM	0.250	0.149	0.043	0.179	0.005	0.130	0.019	0.006	0
P Value	0.9262	<0.0001	<0.0001	0.0065	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

$^{a-c}$: Means within a column without a common superscript differ significantly (P≤0.05). Treatments: 1= (1.5°C), 2= (2°C), 3= (3.5°C), 4= (5°C), control= (20°C).

Table 4 - Effect of low temperature on embryo malposition

Treatment	Head between thighs	Head in the small end of egg	Head under left wing	Head not direct toward air cell	Beak above right wing	Total
1	0.39^{a}	0.15 ^{c}	0.06 ^{b}	0 ^{b}	0.50 ^{a}	1.10 ^{a}
2	0.22 ^{b}	0.48 ^{b}	0.00 ^{d}	0 ^{b}	0.37 ^{b}	1.07 ^{a}
3	0.22 ^{b}	0.48 ^{b}	0.06 ^{b}	0 ^{b}	0.13 ^{c}	0.89 ^{b}
4	0.17 ^{c}	0.58 ^{a}	0.12 ^{a}	0.02 ^{a}	0.00 ^{d}	0.89 ^{b}
Control	0.06 ^{d}	0.16 ^{c}	0.04 ^{c}	0 ^{b}	0.00 ^{d}	0.26 ^{c}
SEM	0	0.015	0	0	0	0.015
P Value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

$^{a-c}$: Means within a column without a common superscript differ significantly (P≤0.05). Treatments: 1= (1.5°C), 2= (2°C), 3= (3.5°C), 4= (5°C), control= (20°C).

To cite this paper: Hussain A, Bilal M, Habib F, Gola BA, Muhammad P, Kaker A, Yousaf A, Khalil R (2019). Effects of low temperature upon hatchability and chick quality of Ross-308 broiler breeder eggs during transportation. *Online J. Anim. Feed Res.*, 9(2): 59-67. www.ojafr.ir

Table 5 - Effect of low temperature on embryo deformity and disorders

Treatment	Exposed brain	Without eyes	4 legs	Deformed beak	Total
1	0.22[a]	0[c]	0.13[a]	0.90[c]	1.25[c]
2	0.11[c]	0.40[b]	0.05[ab]	2.48[a]	3.04[b]
3	0.09[d]	0[c]	0[b]	0[d]	0.06[d]
4	0.13[b]	1.33[d]	0[b]	2.36[b]	3.82[a]
Control	0.02[c]	0[c]	0[b]	0[d]	0.02[d]
SEM	0	0.007	0.03	0	0.033
P Value	<0.0001	<0.0001	0.0396	<0.0001	<0.0001

[a-c]: Means within a column without a common superscript differ significantly (P≤0.05). Treatments: 1= (1.5 °C), 2= (2 °C), 3= (3.5 °C), 4= (5 °C), control= (20 °C).

Table 6 - Effect of low temperature on cull chicks

Treatment	String navel	Button navel	String + button	Body cavity full	Red hock	dehydrated	Stubby down	Dirty chicks
1	1.77[e]	20.79[a]	22.56[a]	58.09[d]	4.29[a]	7.82[b]	4.08[d]	3.15[a]
2	6.27[c]	12.79[b]	19.07[b]	56.29[e]	4.18[a]	4.99[d]	12.98[c]	2.49[b]
3	12.53[b]	6[c]	18.53[b]	76.36[a]	0[d]	5.10[c]	0[e]	0[c]
4	14.47[a]	0[e]	14.47[e]	62.63[b]	1[c]	4.53[e]	17.37[a]	0[c]
Control	5[d]	2[d]	7[d]	61.56[c]	1.43[b]	11[a]	16[b]	0[c]
SEM	0.172	0.264	0.292	0.323	0.133	0	0.082	0.038
P Value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

[a-c]: Means within a column without a common superscript differ significantly (P≤0.05). Treatments: 1= (1.5 °C), 2= (2 °C), 3= (3.5 °C), 4= (5 °C), control= (20 °C).

DISCUSSION

The vitelline membrane contains many proteins, carbohydrates, and lipids. Eggs stored for 6 months at temperatures ±1 °C experience weight loss, loss of nitrogen, and changes in the chemical composition of vitelline (Trziszka et al., 2010). The vitelline membrane loses its natural state (selective nature) between 20 and 30 days of storage (Smoliska et al., 2011). After 6 weeks eggs stored at 4 °C showed a decrease in the elastic properties of the vitelline membrane; this resulted in problems that facilitated the rupture of the vitelline membrane (Jones et al., 2005).

Storage time and temperature are 2 factors that affect the vitelline membrane. The strength of the vitelline membrane decreases with increasing storage time (Khan et al., 2016). This may allow nutrients in the yolk to become available to any microorganism present in the albumen (Akyurek et al., 2009). Wrinkling of the vitelline membrane in sub-zero temperatures, under cold stress, is in line with this finding. Egg water loss during storage has a positive effect on hatchability (Walash et al., 2015). The percentage of egg weight loss between 1 and 19 days in Cobb 500 and Ross 308 strains is 10.9% and 12.9%, respectively (Hussain et al., 2018). Age of the flock (before 44 weeks) does not affect egg weight loss, but the effect of strain is significant (Abudabos et al., 2010). Egg weight loss reaches 12% up to 18 days, and as the altitude increases, the drop in egg weight increases (Cobb-Vantress, 2014). According to Aviagen, egg weight loss should be 12% from the time eggs set in the incubator up to transfer; under these conditions hatchability and chick quality are ideal. If eggs are stored for more than 6 days, egg weight loss will reach 11.5% (ROSS, 2016). In the current study, egg weight loss in control eggs was 12.16%, which conforms to the observations of Aviagen and Cobb-Vantress. When compared with the control group, cold stress caused an increase in the egg weight loss. However, there was not a significant difference between the treatments. An increase in exploder eggs can be caused by a dirty nest, egg laying on the floor, egg washing, using dirty egg cleaner or sandpaper, high levels of dust in the nest, transportation and cold conditions, sweating eggs, spraying water on eggs, broken eggs, hand contamination of egg handlers, contamination of the setter floor, air filters, and the moisture supply system (Wilson, 2014). The current study demonstrated that cold stress is another factor affecting the percentage of exploder eggs. Early hatch in the incubator can be attributed to small eggs, differences between breeds, as well as high temperatures and low humidity in the incubator (Wilson, 2014). In the present study, cold stress especially at temperatures below zero increased the rate of early hatched chicks. Chick weight is affected by egg weight (Tona et al., 2014, Wilson, 2011). A decrease in temperature (from 37.8 to 36.6 °C) during incubation resulted in an increase in chick body weight, from 39 to 40 g, and yolk sac weight, from 3 to 4.3 g (Joseph et al., 2016). These findings are consistent with the findings of others; there were overweight chicks in treatment 1 and a lack of yolk uptake and inappropriate yolk usage. Chick yield or chick weight-to-egg ratios are normally between 66%

To cite this paper: Hussain A, Bilal M, Habib F, Gola BA, Muhammad P, Kaker A, Yousaf A, Khalil R (2019). Effects of low temperature upon hatchability and chick quality of Ross-308 broiler breeder eggs during transportation. *Online J. Anim. Feed Res.*, 9(2): 59-67. www.ojafr.ir

and 68% (Cobb-Vantress, 2014). This ratio in fresh eggs (stored for a short period of time) was between 67% and 67.5% (Tullett, 2009). Other research reported chick yield at 36 weeks at 73.1% (Abudabos et al., 2010). In the current study, the chick weight-to-egg ratio was higher in treatment 1. Improper use of nutrients inside the egg and yolk sac prevents chicks from growing enough. A temperature decrease from 37.8 to 36.6°C during incubation reduced chick length from 16.8 to 16.3 cm (Joseph et al., 2016). Chick length in this experiment was affected by cold stress; reducing the temperature during transport also reduced chick size. Percentage hatchability and hatch of fertile eggs (Ross 308, 33 weeks of age) were 82.8% and 86.9%, respectively (Abudabos et al., 2010). A temperature decrease from 37.8 to 36.6°C in incubation caused a reduction in hatchability (salable chicks) from 87% to 81% (Joseph et al., 2016). In the current study, reducing the temperature level reduced the hatch of fertile eggs and hatchability. The rate of cull chicks was 0.97%. Although it was 0.68% at 38 weeks and 0.42%-0.67% at less than 43 weeks of age, the highest rate was 1.49% at 63 weeks (Cobb 500) (Leonor et al., 2012). In the current study, the percentage of cull chicks and hatchery waste increased as the temperature was reduced. Uniformity of flock is the percentage of chicks within ±10% (Ensminger et al., 2010) or ±15% (Cobb-Vantress, 2014) of the average body weight at a certain age. The goal is 80% uniformity in a flock. Uniformity is affected by many factors including egg size, shell quality, genetic variation of parents, flock density, quantity or quality of feed consumed, parasites, environmental conditions (nest temperature), photoperiod programs, feed restriction programs, broiler breeder body weight (Abbas et al., 2010), incubator type or model (single stage or multistage), incubation conditions, duration of egg storage, initial egg weight, chick room conditions, condition of trucks and transportation system, variation between eggs (non-uniform chicks produced from small or large eggs), mixing eggs from young and old flocks, mixing eggs from different strains, storage periods, different patterns of egg storage, ventilation, non uniform setter and hatcher conditions, diseases, and stress (Wilson et al., 2014). Hatchability in flocks with 55%-59% and 75%-80% uniformity was 69.19 ± 1.93% and 83.93 ± 1.65%, respectively (Abbas et al., 2010). In the current study, cold stress severely reduced uniformity of body weight. Egg contamination rates from 0 to 21 days (in a flock 31-45 weeks of age) were nearly 0.5% (Tullett et al., 2009). Placing cold stress on eggs before incubation significantly increased the number of contaminated eggs and chicks with full body cavity. Cold stress had a significant effect on total embryonic mortality, and with reduction in temperature (below 4 °C) the rate of mortality increased. Reducing the temperature did not affect infertility. Egg shell contamination, nest contamination, ventilation status, and hatchery disinfection can cause increases in embryo mortality (Shane et al., 2010). An increase in the duration of egg storage, fumigation within 12 to 16 h of incubation, high or low temperatures, egg damage during transportation (jarring), illness, an old or aging flock, contamination, drugs, and pesticides are factors that can increase embryonic mortality (Wilson et al., 2014). Early embryonic mortality (33 weeks, Ross 308) was 2.5% (Abudabos et al., 2010). A decrease in incubator temperature from 37.8 to 36.6 °C increased early embryonic mortality from 2.7% to 2.8% (Joseph et al., 2016). Mortality at 12-17 days is caused by inappropriate incubator temperature, moisture, nutritional deficiency (vitamins, phosphorus), and lethal genes (Wilson et al., 2014, Lundy, 1999). Reduction in incubator temperature from 37.8 to 36.6 °C increased embryonic mortality at the middle stage from 0.2% to 0.5% (Joseph et al., 2016). Embryo mortality in the middle stage (33 weeks, Ross 308) was 3% (Abudabos et al., 2010). Cold stress (-2 °C) before the incubation period increased embryo mortality and most mortality reported after the 16th day (wilson et al., 2011). In the current study, we found that early stage embryo mortality in treatment 1 was higher than in other treatments. Eggshell pipping can result from upside-down eggs, setting fumigation at a high concentration, high temperature, high humidity, low turning, old eggs, large-sized eggs, and ventilation problems (wilson et al., 2011). Embryo mortality at the end of incubation (late stage) in a 33 week Ross 308 was 1.1% (Abudabos et al., 2010). A reduction in incubator temperature from 37.8 to 36.6°C increased embryonic mortality at the pipping stage (beak entering air cell) from 0.8% to 1.4% and at external pipping (tip of beak puncturing the eggshell) from 0.9% to 2.4% (Joseph et al., 2016). The incidence of ECV is increased by high incubator temperatures, inheritance, and lethal genes (Wilson et al., 2014).

In the current study, cold stress significantly increased ECV rates. The quality of chickens during incubation depends on various factors such as maternal age, flock status, length of storage and storage conditions, and incubation conditions (Tona et al., 2005). Inside the egg a normal embryo must position itself with the head (beak) under the right wing and placed directly into the air cell. Usually 1%-2% of chickens have malpositions and deformity, and these disorders are observed in the last week of incubation. In this study, malpositions occurred in 1.2%-1.8% of chicks (average 1.5%). Most embryos have a type of malposition that prevents the use of oxygen and they die in the shell (dead in shell); only a few of these embryos are able to leave the shell. Type 6 malpositions account for 48% of malpositions (beak above right wing); type 5 (feet on the head), 20%; type 1 (head between the thighs), 12.5%; type 2 (head towards the end thin egg), 7.5%; type 3 (head under left wing), 7.5%; and type 4 (head not directed toward air cell), 4.5% (Butcher et al., 2009). Cold stress significantly affected total malpositions. Most malpositions (1.10%) occurred in treatment 1. A decrease in temperature towards -2 °C increased the incidence of malpositions. One of the important factors affecting the occurrence of malpositions is insufficient egg weight loss in the incubator (Butcher et al., 2009). In the present study, the rate of egg weight loss in all treatments was higher than in the control group

To cite this paper: Hussain A, Bilal M, Habib F, Gola BA, Muhammad P, Kaker A, Yousaf A, Khalil R (2019). Effects of low temperature upon hatchability and chick quality of Ross-308 broiler breeder eggs during transportation. *Online J. Anim. Feed Res.*, 9(2): 59-67. www.ojafr.ir

and the total rate of malposition was associated with it. Deformity or abnormalities prevent embryos from leaving the eggs. The rate of deformity was 0.22%-0.3%. Most abnormalities occur between 15 and 21 days of incubation and include brain hernia (29%), beak deformity (27%), failure to develop eyes (25%), 4 legs (10%), lack of upper beak (8%), and twisted legs (1%). High incubation temperatures cause abnormalities in the brain and eyes, while low temperatures affect chicken growth. At normal hatchability status (85%) the rate of deformity does not exceed more than 0.3% (Butcher et al., 2009). In our experiment, cold stress caused a significant increase in the rate of deformity in treatment groups compared to the control. Inheritance and viral infection cause cross beaked chickens and chickens without eye(s); these and other malformations are also caused by high temperatures, problems in egg handling, or delay in hatch time due to low temperatures during incubation (Cobb-Vantress, 2014). We observed that cold stress increased dirty chicks, red hock, button navel, and total string and button navel. In conclusion, cold stress during the delivery of eggs from farm to hatchery affects the performance of the developing embryo during incubation; cold stress also impacts chick quality at the time of hatch and at later stages of life.

CONCLUSION

The current study is concluding that cold stress had a significant effect on percentage of egg weight loss and minimal egg weight loss occurred in the control group. The percentage of exploders and early hatched chicks and chick weight were higher in the below 3°C temperature treatment than the other groups. Cold stress had played a vital effect upon chick length, hatchability, and the hatching of fertile eggs. The effects of cold stress on chick yield and body weight uniformity were significant. The effect of cold stress on hatchery by product efficiency was important, but it did not affect fertility. Cold stress also had considerable effects on early, middle, and late mortality; total embryo mortality; and exposed brain. Ectopic viscera and most mortality were observed in below 4°C treatments. Total percentages of malpositions and deformity and egg contamination at (first stage) and (second phase) were affected by cold stress. Cold stress also had an important impact on the number of cull chicks; percent of string navel, button navel, total string, and button; omphalitis; full body cavity; red hocks; dehydration; dirty chickens; and stubby down. Cold stress affects performance during incubation and overall chick quality.

DECLARATIONS

Corresponding Author
E-mail: dr.adnan011@gmail.com

Author's contribution
Dr. Ahmad Husaain and Dr. Muhammad Bilal both are the main researcher of this research work carried out. Dr. Faiza Habib was research coordinator, Dr. Barkat Ali Gola and Dr. Peer Muhammad assisted in results analysis, Dr. Amanullah Kaker and Dr. Rabia Khalil was advisor in treatment line and proposal making, while Dr. Adnan Yousaf helped in correspondence.

Competing interests
The authors declare that they have no competing interests.

REFERENCES

Abbas SA, Gasm Elseid AA, Ahmad MK A (2010) Effect of body weight uniformity on the productivity of broiler breeder hens. Int. J. Poult. Sci., 9: 225-230.

Abudabos A (2010). The effect of broiler breeder strain and parent flock age on hatchability and fertile hatchability. Int. J. Poult. Sci., 9: 231-235.

Akyurek H, and Okur AA (2009) Effect of storage time, temperature and hen age on egg quality in free range layer hens. J. Anim. Vet. Adv., 8: 1953-1958.

Butcher GD, and Nilipour AH (2009) Chicken embryo malpositions and deformities. Reviewed. University of Florida, USA. 129: 1-3.

Cobb-Vantress Inc. (2014) Guide Management Hatchery. Revised (Arkansas).

Deeming DC (1989) Failure to turn eggs during incubation: development of the areavasculosa and embryonic growth. J. of Morphol., 201: 179-186.

Ensminger ME, Oldfi eld JE, Heinemann WW (2004). Feeds and nutrition. The Ensminger Publishing Company. USA. 593-666.

Ensminger ME (2010) Poultry Science. Second Edition. The Interstate Printers and Publishers, Inc., Danville, Illinois, USA. 325-326.

To cite this paper: Hussain A, Bilal M, Habib F, Gola BA, Muhammad P, Kaker A, Yousaf A, Khalil R (2019). Effects of low temperature upon hatchability and chick quality of Ross-308 broiler breeder eggs during transportation. *Online J. Anim. Feed Res.*, 9(2): 59-67. www.ojafr.ir

Hangalapura BN, Nieuwland MG, Buyse J, Kemp B, Parmentier HK (2004) Effect of duration of cold stress on plasma adrenal and thyroid hormone levels and immune responses in chicken lines divergently selected for antibody responses. Poult. Sci., 83: 1644-1649.

Hussain A, Yousaf A, Mushtaq A (2018). Prevalence of mycoplasma gallisepticum in ross308 broiler breeder through the contrast of serological assessments in Pakistan. J Dairy Vet Anim Res. 7(1): 00185.

Jabbar A, Yousaf A. (2017). Effect of age wise incubation programme on broiler breeder hatchability and post hatch performance. Online J. Anim. Feed Res., 7(1): 13-17.

Jones DR, Musgrove MT (2005) Effects of extended storage on egg quality factors. Poult. Sci., 84: 1774-1777.

Joseph N, Lourens SA, Moran ET (2016) The effects of suboptimal eggshell temperature during incubation on broiler chick quality, live performance, and processing yield. Poult. Sci., 85: 932-938.

Jull M, McCartney AMG, Ibiary HM (2001) Hatchability of chicken and turkey eggs held in freezing temperatures. Poult. Sci. 26:545-546.

Khan A, Rind R, Shoaib M, Kamboh AA, Mughal GA, Lakho SA, Malhi KK, Nizamani AR, Yousaf A (2016). Isolation, identification and antibiogram of Escherichia coli from table eggs. J. Anim. Health Prod. 4(1): 1-5

Klasing KC.(2003) Comparative avian nutrition Cambridge University Press. UK. 277-299.

Kucuk O, Sahin N, Sahin K, Gursu MF, Gulcu F, Zcelik MO, Issi M (2003) Egg production, egg quality, and lipid peroxidation status in laying hens maintained at a low ambient temperature (6 °C) and fed a vitamin C and vitamin E-supplemented diet. Vet. Med., 48: 33-40.

Leonor H, Reis M, Chaviro Soares M (2012). The effect of candling on the hatchability of eggs from broiler breeder hens. J. Appl. Poult. Res., 2: 142-146.

Lundy H (1999) A review of the effects of temperature, humidity, turning and gaseous environment in the incubator on the hatchability of the hen's egg (Chapter 9). In: Carter,

Taylor J N, Schober TJ, Bean SR (2006). Novel food and non-food uses for sorghum and millets. Cereal Science, 44: 252–271

Freeman TC, Eds BM. The Fertility and Hatchability of the Hen's Egg. Oliver and Boyd, Edinburgh, UK.; 143-176.

Molenaar R. (2009). Evaluation of chick quality: which method do you choose? Hatchtech Incubation Technology. The Netherlands.

Nakage ES, Cardozo JP, Pereira GT, Queiroz SA, Boleli IC (2003). Effect of temperature on incubation period, embryonic mortality, hatch rate, egg water loss and partridge chick weight (Rhynchotus rufescens). Rev. Bras. de Ciên. Avíc., 5: 1-5.

ROSS (2016). Parent stock management manual. Aviagen Limited Newbridge, Midlothian, EH28 8SZ, Scotland, United Kingdom.

Shane S (2010). Promoting chick quality and liveability. Am. Soy Assoc. Tech. Bulletin, 43: 1-3.

Siegel HS (2003). Stress, strains and resistance. Brit. Poult. Sci., 36: 3-24

Smoliska T, Trziszka T (2011). The vitelline membrane: dynamics of cholesterol metabolism hen eggs Food. Chem., 8: 215223.

Taylor LW (2003). The care of hatching eggs before incubation (Chapter 4). In: Taylor, L.W. Fertility and Hatchability of Chicken and Turkey Eggs. Greenworld Publisher, India. 150-177.

Tona K, Bruggeman V, Onagbesan O, Bamelis F, Gbeassor M, Decuypere E (2005) Day old chick quality: relationship to hatching egg quality, adequate incubation practice and prediction of broiler performance. Avi. & Poult. Biol. Rev. 16: 109-119.

Tona K, Onagbesan O, De Ketelaere B, Decuypere E, Bruggman V. (2014). Effect of age of broiler breeders and egg storage on egg quality, hatchability, chick quality, chick weight and chick posthatch growth to forty-two days. J. Appl. Poult. Res., 13: 10-12.

Trziszka T, Smoliska T (2010). Chemical characterization of the vitelline membrane of hen eggs. Food Chem., 8: 61-70.

Tullett S (2009). Investigating hatchery practice. Ross Tech. Scotland, United Kingdom.

Walash TJ, Rizk RE, Brake J (2015). Effects of temperature and carbon dioxide on albumen characteristics, weight loss, and early embryonic mortality of long stored hatching eggs. Poult. Sci., 74: 1403-1410.

Wilson R, Runde A, Zhu Min Yan Vogelsang L, Leptin M. (2001). Mechanisms of FGF signalling during Drosophila morphogenesis. *Biol. Cell* 93(3-4): 147.

Wilson HR (2011) Physiological requirements of the developing embryo (Chapter 9). In: Tullet, S.G. Avian Incubation. Butterworth-Heinemann; London. 145-156.

Wilson HR (2014) Hatchability problem analysis, CIR1112, University of Florida, Gainesville. USA.

Wilson HR (2014) Interrelationship of egg size, chick size, posthatching growth and hatchability. World. Poult. Sci., J. 47: 5-20.

Yousaf A, Jabbar A, Ditta YA (2017). Effect of pre-warming on broiler breeder eggs hatchability and post-hatch performance. J. Anim. Health Prod. 5(1): 1-4.

Yousaf A, Jabbar A, Laghari IH, Abbas M (2017). Effect of incubation duration on broiler breeder eggs hatchability and post-hatch performance. J. Anim. Health Prod. 5(4): 127131.

Yousaf A, Rubab F, Shahnawaz R, Jamil T, Iqbal T, BiBi N, Haider I. (2016). Impact of semen quality of Aseel chicken on induced molting. Online J. Anim. Feed Res., 6(6): 130-132.

To cite this paper: Hussain A, Bilal M, Habib F, Gola BA, Muhammad P, Kaker A, Yousaf A, Khalil R (2019). Effects of low temperature upon hatchability and chick quality of Ross-308 broiler breeder eggs during transportation. *Online J. Anim. Feed Res.*, 9(2): 59-67. www.ojafr.ir

Yousaf A, Shahnawaz R, Jamil T, Mushtaq A (2018). Prevalence of coccidiosis in different broiler poultry farms in Potohar region (distract Rawalpindi) of Punjab- Pakistan. J Dairy Vet Anim Res., 7(3): 87–90.

Yousaf A. (2016). Impact of gender determination through vent sexing on Cobb-500 broiler performance and carcass yield. Online J. Anim. Feed Res., 6(6): 125-129

Zamani P (2010). Statistical designs in animal science. Bu-ali Sina University Press, Hamadan, Iran. 101-123.

To cite this paper: Hussain A, Bilal M, Habib F, Gola BA, Muhammad P, Kaker A, Yousaf A, Khalil R (2019). Effects of low temperature upon hatchability and chick quality of Ross-308 broiler breeder eggs during transportation. *Online J. Anim. Feed Res*., 9(2): 59-67. www.ojafr.ir

2019 SCIENCELINE

Online Journal of Animal and Feed Research

Volume 9, Issue 2: 68-85; March 25, 2019 ISSN 2228-7701

A REVIEW ON EQUINE WOUND MANAGEMENT AND HEALING PROCESS

Addisu Mohammed SEID[1]✉ and Mastewal BIRHAN[2]

[1]*Department of Veterinary Clinical Medicine, College of Veterinary Medicine and Animal Sciences, University of Gondar, Ethiopia*
[2]*Department of Veterinary Paraclinical studies, College of Veterinary Medicine and Animal Sciences, University of Gondar, Ethiopia*

✉*Supporting Information*

REVIEW
PII: S222877011900010-9
Received: October 09, 2018
Revised: March 18, 2019

ABSTRACT: Skin lacerations and other traumatic injuries of the integument are frequently seen in equine practices and range from relatively minor cuts to severe, potentially debilitating injuries. The early stages of wound healing (inflammatory or debridement stage) that are clean or clean contaminated are the best candidates for primary or delayed primary closure. Wounds that are in the later stages of healing or are contaminated or infected heal best by second intention. Management is dictated by the nature and size of the wound, the area of the body on which the wound occurs, and several aspects of wound healing. The age of the wound, integrity of the local blood supply, degree of contamination, location of the injury, skin loss, and local tissue damage must all be considered when deciding on the most appropriate method for managing a particular wound. In addition to biologic factors, the physical size of equine patients and the environment in which they are kept present unique management challenges not encountered in the treatment of soft tissue injuries in other species. Appropriate wound care is always a balance between improving the wound environment and harming the cells that are integral to the healing process. Consequently, the veterinarian must carefully weigh the benefits and the detriments of any particular action, not only for the immediate results but also for the long-term healing process. Understanding the principles and limitations of reconstructive procedures, adhering to the basic principles of equine wound management, and providing appropriate postoperative care all contribute to a successful outcome.

Keywords: Debridement, Equine, Inflammation, Management, Wound

LIST OF ABBREVATIONS

ATP	Adenotri Phosphate
DACA	Drug Administration and control Authority
ECM	Extracellular Matrix
EGT	Exuberant Granulation Tissue
FAOSTA	Food and Agricultural Statistics
HBO	Hyperbaric Oxygen
L	Liter
MMPs	Matrix Metalloprotienases
PA	Plasminogen Activator
PCV	Packed Cell Volume
PMNs	Polymorphonuclear Leukocytes
PSI	Pounds Per Square Inch
SIS	Small Intestinal Submucosa
SPP	Species
VEGF	Vascular Endothelial Growth Factor

INTRODUCTION

More than 72% of the world's horse population is found in developing countries specifically kept for draft purpose (Swann, 2006). Ethiopia has more than 6 million donkeys, the second largest donkey population in the world next to China, 1.9 million horses and over 350,000 mules (FAOSTAT, 2012). Equines are important animals to the resource-poor communities in rural and urban areas of Ethiopia, providing traction power and transport services at low cost (Dinka et al., 2006).

To cite this paper: Seid AM and Birhan M (2019). A Review on Equine Wound Management and Healing Process· *Online J. Anim. Feed Res.*, 9(2): 68-85. www.ojafr.ir

In Ethiopia, the rugged terrain characteristics, absence of well-developed modern transport networks and the prevailing low economic status of the community necessitate the use of equines for transportation (Mengistu, 2003). Wound is an open mechanical injury of the skin (epidermis), underlying tissues and organs. It is characterized by pain, gaping bleeding functional disturbance. The most common cause of wounds in working equine are over loading, accidents, improper position of load predisposing to falling, hyena bites, donkey bites, injuries inflicted by horned Zebu (DACA, 2006). Some hobbling methods, inappropriate harnesses or yokes that may be heavy and ragged, long working hours may cause discomfort and inflict wounds (Mekuria et al., 2013).

Wounds are one of the primary welfare concerns of working equids (Sells et al., 2010). The type of wound in working donkeys includes tissue damage with or without blood/exudates/ pus, abscess formation, or any secondary bacterial complication. Bites (lacerated wounds) will be identified by irregular edges with underlying tissues removed as well as hemorrhage (Sevendsen, 2008).

Wounds can be either traumatic or surgical in origin; both types can fail to heal and become chronic although traumatic wounds are more commonly affected by healing difficulties. The incidence and prevalence of traumatic wounds in equine is considered to be high (Singer et al., 2003) and a high percentage become chronic, adding more complexity to wound healing management strategies. Chronic wounds in horses have a similar pathophysiology to human chronic wound (Cochrane et al., 2003). Treatment methods that are employed in the management of wounds focus on rapid and efficient evaluation, scrupulous, aseptic surgical techniques, and conscientious and prolonged aftercare. Appropriate antibiotic treatment regimens are routinely employed when the wound is at risk of becoming infected or is known to be infected (Griffiths et al., 2003). There for, the aim of this review paper is to clarify the types of wound in equine and what are the processes and the phase to heal it.

GENERAL OVER VIEW ABOUT WOUND

Initial preparation

After the equine has been restrained appropriately and the area has been desensitized with local anesthetic, the clinician decides whether to treat the wound with aseptic technique or if a simple clean technique is adequate. If there is a possibility of cross-contaminating a wound while preparing and exploring, aseptic technique should be used. The next step is to clip the hair over and around the wound site. To minimize the amount of clipped hair that gets into the wound, it can be covered with water-soluble, sterile, lubricating gel prior to clipping. It is best to put on a pair of examination or sterile gloves, apply the gel onto the fingers, and rub the gel into place. This technique generally ensures that the gel will remain in contact with the wound bed. As the hair is clipped, it is trapped in the gel. The gel with the trapped hair can simply be rinsed from the wound with water or saline. Only when the entire wounds area has been clipped and prepared can the clinician begin to fully appreciate the complexity of the wound. In many cases, seemingly insignificant wounds had invaded synovial structures, and because appropriate aggressive therapy was not instituted, the horse was eventually euthanized (Auer and Stick, 2006).

Wound assessment

Wound assessment is of the most important steps in wound management. More repaired wounds fail because of improper preparation and assessment than because of improper therapy. Properly preparing the wound provides an accurate assessment of the wound and deeper structures. Wound preparation begins with placing the animal in allocation and environment that will allow the clinician to best determine the status of the wound (Auer and Stick, 2006).

Wound classification

Wounds are typically classified on the basis of degree of contamination. Clean wounds, usually seen only in surgical situations, are not infected and do not involve the respiratory, alimentary, or urogenital tract. Clean contaminated wounds, generally seen in surgical situations, involve the lumen of the respiratory, alimentary, or urogenital tract. Contaminated wounds are generally traumatic in nature and may have gross contamination and necrotic debris. Infected wounds generally involve large numbers of bacteria, inflammation, edema, and suppuration (Knottenbelt et al., 1998).

Debridement

Debridement is the process of removing dead (necrotic) tissue or foreign material from and around a wound to expose healthy tissue. Necrotic tissue and bacterial infection are major roadblocks to effective and cosmetic wound healing. An open wound or ulcer cannot be properly evaluated until the dead tissue or foreign matter is removed. Wounds that contain necrotic and ischemic (low oxygen content) tissue take longer to close and heal. This is because necrotic tissue provides an ideal growth medium for bacteria, especially for Bacteroides spp. and Clostridium perfringes that causes the gas gangrene so feared in military medical practice. Though a wound may not necessarily

To cite this paper: Seid AM and Birhan M (2019). A Review on Equine Wound Management and Healing Process· *Online J. Anim. Feed Res.*, 9(2): 68-85. www.ojafr.ir

be infected, the bacteria can cause inflammation and strain the body's ability to fight infection. Debridement is also used to treat pockets of pus called abscesses. Abscesses can develop into a general infection that may invade the bloodstream (sepsis) and lead to amputation and even death. Burned tissue or tissue exposed to corrosive substances tends to form a hard black crust, called an eschar, while deeper tissue remains moist and white, yellow and soft, or flimsy and inflamed. Eschars may also require debridement to promote healing. The most common types of debridement are sharp, mechanical, chemical, and autolytic (Falanga and Harding, 2002).

Sharp debridement. Sharp debridement refers to the use of forceps, scissors, or a scalpel to remove devitalized tissue, debris or other foreign materials from a wound bed (Ayello et al., 2008). After surgical debridement, sharp debridement is the most aggressive form of debridement available to clinicians, and also the most rapid. Vigorous and repeated sharp debridement of necrotic tissue and debris is considered the standard of care for many patients with open wounds. The technique is indicated in wounds presenting with significant necrosis, callus, advancing cellulitis or sepsis, or thick adherent eschar. Chronic wounds tend to require repeated debridement because of the likely re-emergence of necrotic tissue and bio burden in the wound bed. Sharp debridement has a number of contraindications, and should not be used when area cannot be adequately visualized or when the material to be debrided is unidentified. The technique should be used with caution in patients who are immunosuppressed, thrombocytopenic, or receiving anticoagulant therapy. As sharp debridement can be stressful for the patient, the procedure should be halted if the patient experiences uncontrolled pain or if there is extensive bleeding. Importantly, the procedure should not proceed if the clinician fatigues, and should only be undertaken if sufficient time is available to both clinician and patient (Myers, 2008).

Mechanical debridement. Mechanical debridement is more traumatic, and un fortunately it is probably the most commonly used method in equine. It can be performed using woven gauze, lavage, wet-to-wet, or wet-to-dry dressings. Mechanical debridement can be a useful tool, and it can be beneficial in the healing process, but it can be very traumatic. Often, when using gauze, too much pressure is applied and trauma to the wound bed ensues. Only gentle pressure should be used. If more aggressive debridement is necessary, sharp debridement should be performed. Woven gauze provides mechanical debridement characteristics superior to those of nonwoven gauze. Lavage can be used successfully for mechanical debridement. It involves two critical components, the selection of a non-cytotoxic cleansing solution and the delivery of that solution to the wound surface with appropriate pressure and volume to wash away the necrotic debris without pushing it further into the tissue planes of the wound (Ovington, 2001). The pressure should be between 10 and 15 pounds per square inch (psi). One way to achieve this pressure is to attach a 19-gaugeneedle or catheter to a 35-ml syringe (Rodeheaver, 2001). Many devices are available. Showerheads are safer and more effective than single-jet lavage systems. Therefore, devices that produce high pressures (e.g., the Water Pik) should not be used. A simple, gentle, low-pressure lavage system can be made by using a 16-gauge needle to punch four to eight holes in the cap of a 1-L bottle and then squeezing saline out. The fluids most commonly used in veterinary medicine are dilute antiseptics. Saline has been shown to be effective in reducing bacterial counts in an infected wound but to have no effect on wound healing in clean wounds (Hollander *et al*., 1998). In one study comparing saline and water in an infected wound in a rat model, saline reduced the bacterial counts by 81.6% whereas tap water reduced bacterial counts by 82% (Muscati et al., 1998). However, work by Buffa and coworkers suggests that tap water is very toxic to fibroblasts .When exudate is present, surfactant-based wound cleansers are more effective than saline or poly ionic fluids (Rodeheaver, 2001).

Chemical debridement. Chemical debridement has been used in many different forms in medicine. Dakin's solution, a diluted sodium hypochlorite (bleach) solution, was originally used during World War I. Other chemical agents are hydrogen peroxide, acetic acid, and, more recently, hypertonic saline. Chemical debridement is nonselective and should be reserved for very contaminated wounds. However, although hypertonic saline is good in selected cases, sharp debridement is probably a better technique for removing large amounts of necrotic debris. Hypertonic dressings provide an effective chemical debridement with minimal damage to the wound in the early stages of wound healing (Auer and Stick, 2006).

Autolytic debridement. Autolytic debridement takes advantage of the body's own ability to dissolve dead tissue. The key to the technique is keeping the wound moist, which can be accomplished with a variety of dressings. These dressings help to trap wound fluid that contains growth factors, enzymes, and immune cells that promote wound healing. Autolytic debridement is more selective than any other debridement method, but it also takes the longest to work. It is inappropriate for wounds that have become infected. Autolytic debridement is the least traumatic of these techniques which is achieved by leaving wound fluid (containing white blood cells and enzymes released from dead white blood cells) in contact with the wound bed. The white blood cells and enzymes affect only the dead and necrotic tissue, leaving healthy cells intact for wound healing. This can occur only in moist wounds. When wounds are allowed to dry, autolytic debridement is slowed significantly. Autolytic debridement reduces the bacterial count by allowing access of white blood cells to the wound bed without causing continued trauma to the wound bed. However, sharp debridement should be performed before using autolytic debridement, as the latter technique is ineffective in the presence of large volumes of necrotic material (Harper and Michael, 2001).

1) Non antimicrobial dressings

Collagens, Maltodextrins. Topical dressings such as collagens and maltodextrins are designed for use in the granulating stage of wound repair. They are both hydrophilic and should maintain a moist wound bed. They are available in powder or gel form. If the wound does not have a lot of exudate, it is probably best to use the gel form to prevent drying out of the wound bed .Maltodextrins may provide nutrition to the wound bed (Purna and Babu, 2000).

Biologic Dressings. Various substances have been used recently as biologic dressings. These dressings are intended to provide a framework over which other cells migrate, and as a stimulant to those cells to form the tissue that is desired. Some of the more commonly used biologic dressings include porcine small intestinal sub mucosa (SIS), porcine bladder basement membrane, equine amnion, and various skin products. The porcine small intestinal sub mucosa and bladder products are not rejected by the host as other xenografts. From clinical experience, they appear to be best if they are kept moist. In a recent study, SIS dressing was applied to fresh and chronic wounds. Compared with similar wounds treated without SIS, exuberant granulation tissue was reduced and drainage was facilitated, which reduced wound exudation and improved epithelialization. Overall, wound healing was faster and costs for bandages and hospitalization were reduced (Dressel et al., 2004). Other studies have shown that the use of equine amnion reduces wound retraction and granulation tissue formation while improving epithelialization. Amnion has been shown to be beneficial as a non-adherent dressing in skin grafting (Goodrich et al., 2000).

Gauze. The use of gauze to dress and bandage wounds has its origins in ancient times, having been firmly established by the fifth century and is still in use today. The term 'gauze' represents two types of bandaging material: woven gauze is the 100% natural cotton cloth that we are most familiar with. Non-woven gauze refers to more modern, synthetic dressings made of rayon or synthetic fiber blends (Jones, 2006). Woven gauze is problematic in dressing and packing wounds as it sheds fibers when cut and may leave debris in the wound bed when removed. It is also absorbent and tends to stick to the wound, resulting in trauma upon removal as it quickly dries out the wound, becoming trapped within the eschar. Until recently, this was considered advantageous as a dry wound was considered optimal for healing and the removal of the embedded dry eschar was seen as a form of physical debridement. The perception that a wound was best healed under dry conditions persisted from the times of Hippocrates until relatively recent research promoted the maintenance of a moist wound environment (Eglasein, 2001). Gauze strips soaked in antibiotics such as EUSOL, proflavin or chlorhexidine were used to pack wounds to prevent closure and promote granulation from the wound base. This was subsequently changed to salinesoaked gauze because the use of antibiotics in this fashion was thought to be potentially cytotoxic. Saline was employed as a hypertonic solution but quickly dried out, resulting in painful removal (Queen and Steed, 2004). Today, woven gauze is seen as a 'wet to dry' dressing and utilized in a range of wound care strategies. Despite its non-selective mode of physical debridement, trauma to the wound bed and resultant pain, it is still the most utilized wound dressing in the world (Steed, 2004). It is also utilized as a vehicle for antimicrobial agents but presents complications such as degradation or inactivation of the antimicrobial agent upon exposure to the high protein levels within wound fluid and lateral bacterial migration into the wound bed within the moist environment. Factors such as cost, education and the ability to follow best practice are thought to determine selection of wound dressings and may account for continued use of this ancient product, seemingly surpassed by modern dressings (Boateng et al., 2001). Although gauze is commonly used, a range of more appropriate dressings has been available for a number of years. These dressings employ many technological advancements and exhibit qualities considered to be essential in the 'perfect' wound dressing such as: the ability to maintain a moist wound environment while preventing maceration of surrounding tissue; non-adherent and a traumatic; thermally insulating and gas-permeable; antibiotic and non-toxic (Martineau and Shek, 2006).

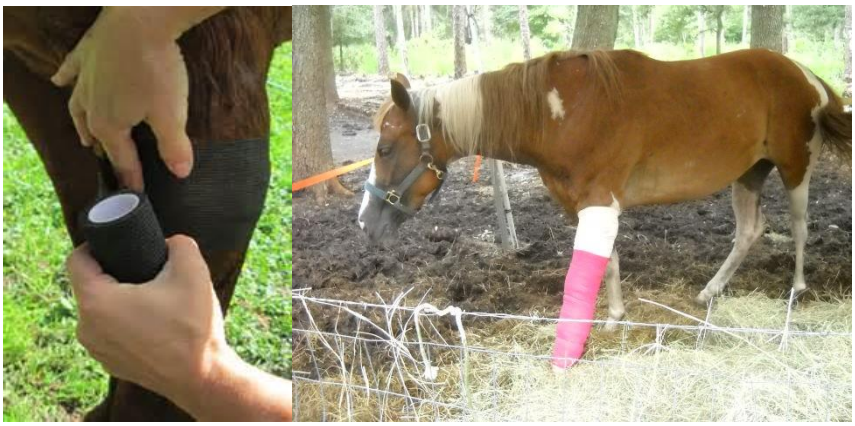


Figure 1. Bandaging

To cite this paper: Seid AM and Birhan M (2019). A Review on Equine Wound Management and Healing Process· *Online J. Anim. Feed Res.*, 9(2): 68-85. www.ojafr.ir

Hydrocolloids: Hydrocolloid dressings, first developed in 1982, consist of an inner layer of hydrophilic gel made from gelatin, pectin, sodium carboxy methylcellulose and poly isobutylene, backed by a film, forming a flexible wafer dressing (Dumnille et al., 2011). These occlusive dressings have been shown to provide a moist, hypoxic wound environment which promotes autolytic debridement. They also allow gas exchange and are semipermeable to vapor but may cause maceration if used on heavily exuding wounds, although it's been suggested that the application of a hydrocolloid dressing may reduce the amount of exudate produced by a wound. Various specific types of hydrocolloid dressings have come to market, but while they differ in size, shape, exudate absorption and intended use, their basic mode of action remains the same (Davies and Rippon, 2006).

Hydrogels: Hydrogels consist of cross-linked polymers such as starch, cellulose or other plant- or animal-derived polysaccharides and contain up to 96% water (Juris et al., 2011). They can provide moisture to dry wounds as well as absorb excess exudate, depending on moisture levels at the wound and are a traumatic when used correctly (Boateng et al., 2001). Hydrogels also facilitate the autolysis of necrotic tissue, and do not support bacterial growth but their use is limited to dry and low exuding wounds as they can cause maceration to surrounding tissues when higher volumes of wound exudate are present (Dumnille et al., 2011). There is also a risk that when used on exuding ischaemic ulcers their use can result in a shift from dry to wet gangrene within the wound. Interestingly, one group has recently reported some active antimicrobial and antifungal properties in a hydrogel dressing based on polyvinyl pyrrolidone/polyethylene glycol polymers (Biazar et al., 2012). Hydrogels are available in a variety of formats such as sheets, gels and beads which enable a tailored application to individual wounds. Hydrogels are utilized for the treatment of burns, chronic ulcers, and surgical wounds and even injected into the spinal column (Macaya and Spector, 2012).

Foams. Polyurethane foam dressings are easy to use and customize as they can be cut to shape and come in a range of absorbencies. They are designed to absorb excess wound exudate while maintaining a moist wound interface and providing thermal insulation. They also prevent maceration of surrounding healthy tissue and facilitate the removal of slough (Varma et al., 2008). A range of foam dressings exists with some incorporating other components to enhance absorbance, control infection or ensure a traumatic removal. These impregnated foams are used for a wide range of applications in both acute and chronic wounds, including post-surgical dressing, application on heavily exudating wounds or for packing deep cavity wounds (Dinah and Adhikari, 2006).

Silicone dressings. Silicone is used either as a contact dressingor as the contact layer within a dressing, for example, Mepilex, a polyurethane foam membrane coated with a soft silicone layer (White, 2005). It is also used as a coating on materials like non-woven polyester nets. In negative pressure therapy, silicone-coated polyester enhances healing rates in sheep models and may assist in the prevention of hypertrophic scarring when combined with pressure therapy. Dressings incorporating soft silicone contact layers adhere to dry skin while remaining non adherent to the wound site, resulting in a traumatic removal and a decreased risk of damage to the wound site upon dressing changes. Silicone can be used on a range of acute and chronic wounds as it is incorporated in many different bandaging strategies (Losi et al., 2012).

Capillary action dressings. Capillary action dressings incorporate an absorbent pad of hydrophilic fibers, typically comprising 80% polyester, 20% cotton fibers between two layers of perforated, permeable, non-woven polyester. Exudate is removed from the wound by capillary action and the excess is spread laterally through the absorbent pad along a capillary pressure gradient, preventing tissue maceration. These dressings decrease bacterial load on the wound surface, assist in debridement and desloughing, but may adhere to wounds with low levels of exudate, resulting in traumatic removal. They are best used in conjunction with a non-adherent contact layer and are not recommended for arterial or heavily bleeding wounds (Deeth et al., 2012).

Odour-absorbent dressings. Odour in a wound is primarily produced from anaerobic bacteria, and whilst the initial line of management should be infection control, it is often advantageous to incorporate an odour-absorbing dressing into the treatment protocol. These dressings use charcoal or activated carbon to absorb odour from the wound and are often used in conjunction with absorbent secondary dressings. The odour-causing molecules are retained by the carbon and charcoal is shown to retain bacteria; when incorporated with antimicrobial agents like silver, antibacterial activity is attained. Efficacy in retaining odour and absorbing wound exudate varies considerably across products (Thomas et al., 2012).

2) Antibacterial dressings

Honey dressings. The use of honey as an antibacterial is well established in modern wound care, with medical-grade honey used in a variety of commercially available dressings. These dressings provide antimicrobial and anti-inflammatory properties through autolytic debridement and maintenance of a moist wound environment while inhibiting bacterial growth, stimulating wound healing and deodorizing the wound, although research trends are mixed in regard to their overall efficacy (Molan, 2006). Honey is bactericidal and antifungal against approximately (Zhong et al., 2010) bacterial strains, both gram-positive and gram-negative, and some yeasts and is often used to control bacterial strains resistant to conventional antibiotics (Cooper, 2008). Antimicrobial action is both mechanical

and enzymatic. Like sugar pastes, honey can inhibit bacterial growth through its osmolality, where the high concentration of sugars causes water to be drawn from the local wound environment. This also maintains a moist wound environment by stimulating fluid transfer from surrounding tissues. Whilst this action dilutes the honey, its antibacterial effects remain (Kwankman, 2012). Honey is applied topically to a wide range of wounds in the form of an ointment, for packing cavities, or impregnated within a hydrogel or alginate dressing. When used as an ointment, the honey will rapidly dilute due to absorption of wound exudate as well as increase in fluidity upon warming to body temperature and may, therefore, require frequent dressing changes in order to maintain efficacy (Molan, 2001).

Iodine dressings. Iodine, a natural halogen, is an antiseptic and available in a range of topical applications. Like all antiseptics, it targets a broad spectrum of bacteria and other pathogens such as fungi, viruses, protozoa and prions through non-specific action (Sibbald et al., 2011). Iodine has been used to prevent and treat infection since the fourth century BCE and debate over its use remains. Although antiseptic use has declined due to the rise of resistant bacterial strains, modern preparations of iodine in managing infection are being explored, but results are conflicting and general consensus remains to be reached (Angel et al., 2008). Iodophors, one such modern formulation, were developed in the 1950s by complexing elemental iodine to a surfactant to improve solubility and reduce cytotoxicity effects. Elemental iodine is cytotoxic against fibroblasts, keratinocytes and leukocytes, thus impeding wound healing. The use of iodophors in modern wound dressings ensures release of lower concentrations of free iodine into the wound exudate. The most widely utilized formulations are povidone-iodine and cadexomer-iodine (Chaikof et al., 2002). The former, while being the most commonly utilized form of iodine in the clinical setting, is not recommended for long-term use or for complex wounds. Indeed, the use of current formulations of povidone-iodine is still contentious. Previous research has shown that clinical concentrations of as little as 1% are cytotoxic to granulocytes and monocytes in vitroand systemic iodine toxicity can occur with povidone-iodine dressings, which typically contain concentrations of 7.5% (Burks, 1998). Studies exploring cadexomer-iodine formulations as a topical application found them to be effective in controlling bacterial load. Subsequent studies in humans and porcine models showed an acceleration of epidermal migration and re-epithelialisation, through upregulation of cytokines like vascular endothelial growth factor (VEGF) (Ohtani et al., 2007). Cadexomeriodine was also found to positively affect healing rates in chronic wounds. Cadexomer-iodine formulations are available as ointments, powders or dressings (hydrogels, ointments gauze, knitted viscose, beads and paste) (Flores and Kingsley, 2007).

Silver dressings. Although the antibacterial action of silver is well established, with silver dressings used in a wide range of infected wounds, their potential cytotoxicity remains an issue (Kim et al., 2012). Ionic, metallic and nanocrystalline forms of silver have been employed as foams, hydrofibres and hydrocolloids. The amount of free silver available to action upon the wound varies from product to product, which impacts upon the effectiveness of the dressing (Jude et al., 2007). Silver ions act upon bacteria by binding and disrupting proteins and nucleic acids through interaction with their negatively charged groups such as thiol groups, carboxylates, phosphates, hydroxyls, imidazoles, indoles and amines as well as stimulating the generation of reactive oxygen species. As a result, cellular changes rapidly occur through a number of mechanisms that result in loss of viability. Investigations have begun only recently on the systemic toxicity of silver nanoparticles. Asharani et al., 2009 investigated the cytotoxicity of silver nanoparticles on human glioblastoma and lung fibroblast cells in vitro. The nanoparticles were found to penetrate into mitochondria and nuclei, interrupting ATP synthesis and resulting in DNA damage. Others have noted the cytotoxic effects of silver nanoparticles on keratinocytes in vitro (Zanette et al., 2011).

Other antibacterial dressings. Antibacterial agents such as chlorhexidine have been incorporated into a wide range of commercially available dressings and washes (Aramwit et al., 2010). Chlorhexidine has been utilized in infection control since the 1950s but has been mostly limited to irrigation and wound cleansing protocols with limited evidence to support efficacy (Cooper, 2004). Chitosan acetate is currently used as a haemostatic dressing in the form of a bandage, but some investigations into its antimicrobial action have shown it can prevent fatal systemic sepsis and control the growth of Pseudomonas aeruginosa and Pseudomonas mirabilis (Dutta et al., 2011).

WOUND CLOSURE TECHNIQUES

After the wound has been successfully débrided, cleaned, and examined, it needs to be closed. The options are suture closure, healing by second intention, skin grafting, or some combination of these to provide a continuous epithelial surface over the wound. The type of closure technique to use depends on what caused the wound, the time from injury, the degree of contamination, the extent of the injury, and potential dead space. That there is a “golden period” of 6 hours from the wounding, after which the wound is considered to be infected, is no longer deemed correct, and it behooves the clinician to examine the wound carefully to determine which of the following techniques to use for wound closure.

1) Primary closure

Primary closure is a technique whereby the wound is closed immediately and completely, using strict aseptic technique. This is the technique most likely to provide the best cosmetic result. Unfortunately, primary closure is

To cite this paper: Seid AM and Birhan M (2019). A Review on Equine Wound Management and Healing Process· *Online J. Anim. Feed Res.*, 9(2): 68-85. www.ojafr.ir

acceptable only in wounds with minimal tissue loss, minimal bacterial contamination, and minimal tension on the wound edges after closure. Regardless of whether primary closure or delayed primary closure is performed, the wound needs to be cleaned and prepared for closure, because excess bacteria in the wound increase the possibility of wound dehiscence. There are many suturing techniques for wound closure and many reviews of suture types for primary closure (Lott-Crumpler and Chaudhry, 2001). For areas of tension, complex suture patterns such as the near-far-far-near, vertical mattress, and horizontal mattress patterns provide more tension reduction than simple patterns such as a simple interrupted or simple continuous pattern. The near-far-far-near suture pattern is most satisfactory, as it provides apposition of the skin edge at the same time as tension relief. Additionally, the mattress patterns can be used with stents to reduce pull-through at the skin–suture interface. A large-diameter suture material should be used if tension exists. One or a combination of four techniques can be used to manage dead space in a wound: suture, meshing, passive or active drains, and pressure bandages. Each technique has benefits and risks that need to be weighed. Suture material, although very useful in wound closure, can also act as a foreign body. Excess suture use (too much suture, too large a diameter, or too many knots) can potentiate infection. Consequently, the clinician should use the smallest-diameter suture material possible, use monofilament, absorbable suture material, and use only surgeon's knots (or interrupted sutures if absolutely necessary when the suture material must be buried). Good surgical technique also benefits the patient, as it reduces trauma to the wound site. If dead space cannot be managed by suture placement, meshing or suction drains (passive or closed) should be employed. However, drains not only allow evacuation of dead space but can also act as a conduit for bacteria to enter the wound. Drains should be left in place only as long as necessary to reduce the possibility of infection. Pressure bandages can and should be used whenever possible in addition to sutures, meshing, or drains, or sometime in place of them to reduce dead space. A properly applied bandage closes dead space without adding any foreign material. However, if a bandage is too tight, the blood supply to the wound may be compromised, leading to wound failure.

2) Delayed primary closure

In a delayed primary closure, the wound is initially treated as an open wound to allow debridement and reduce bacterial contamination. Next, the wound is closed primarily. In some cases, only a portion of the wound can be completely closed. Delayed primary closure is reserved for wounds that have mild to moderate bacterial contamination, minimal tissue loss, and minimal tension on the wound edges after closure. Drains may be placed to evacuate fluid after closure. Delayed wound closure is very useful in the management of abdominal incisions after colonic rupture (Bender, 2003). These studies showed as much as a twofold increase in incisional infection with primary closure of contaminated abdominal wounds. Delayed primary closure after 3 to 5 days did not result in an increased hospital stay. Wounds destined for delayed primary closure should be debrided and cleaned to reduce the bacterial burden. Hypertonic saline dressings, topical antimicrobials, and systemic antimicrobials can be very useful in treating wounds prior to closure.

3) Second-intention healing

Second-intention healing occurs when primary or delayed primary closure cannot be accomplished. In most cases, these wounds have gross contamination and moderate to severe tissue loss that would make closure difficult. They must heal completely through the process of contraction, granulation, and epithelialization (Hohn, 1977).

PHASES OF WOUND REPAIR

1) Acute inflammation

Inflammation prepares the wound for the subsequent reparative phases. It encompasses vascular and cellular responses whose intensity is strongly correlated to the severity of trauma. The injured endothelial cell membrane releases phospholipids that are transformed into arachidonic acid and its metabolites, which mediate vascular tone and permeability as well as platelet aggregation. The first response of the damaged blood vessel is vasoconstriction, lasting 5 to 10 minutes, after which vasodilation ensues and promotes diapedesis of cells, fluid, and protein across the vessel wall into the extravascular space. Coagulated blood and aggregated platelets together form a clot within the defect that, despite providing limited strength to the wound, seals off the injury and prevents further bleeding. The clot also functions as a scaffold through the presence of a large number of binding sites on blood proteins that are recognized by special surface receptors found on migratory inflammatory and mesenchymal cells. Activated platelets are among the earliest promoters of inflammation, via the release of potent chemo attractants and mitogens from their storage granules. These serve as signals to initiate and amplify the reparative phases of healing and are detailed later. Over time, the surface clot desiccates to form a scab that protects the wound from infection. This scab is in turn lysed by plasmin and sloughs along with dead inflammatory cells and bacteria as healing proceeds underneath. The provisional extracellular matrix (ECM) will be replaced by granulation tissue in the next phase of repair. Leukocytes are recruited from the circulating blood pool to the site of injury by the numerous vasoactive mediators and chemo attractants supplied by the coagulation and activated complement pathways, by platelets, by

To cite this paper: Seid AM and Birhan M (2019). A Review on Equine Wound Management and Healing Process· *Online J. Anim. Feed Res.*, 9(2): 68-85. www.ojafr.ir

mast cells, and by injured or activated mesenchymal cells (Singer and Clark, 1999). These signals initiate the processes of rolling, activation, tight adhesion, and finally transmigration of inflammatory cells through the micro vascular endothelium.

Chemo attractants additionally stimulate the release of enzymes by the activated neutrophils; these enzymes facilitate the penetration of the inflammatory cells as they migrate through vascular basement membranes. Cellular influx begins early, and neutrophil numbers progressively increase to reach a peak 1 to 2 days after the injury. The neutrophils act as a first line of defense in contaminated wounds by destroying debris and bacteria through phagocytosis and subsequent enzymatic and oxygen-radical mechanisms. The principal degradative proteinases released by the neutrophils to rid the site of denatured ECM components are neutrophil-specific interstitial collagenase, neutrophil elastase, and cathepsin G. Neutrophil migration and phagocytosis cease when contaminating particles are cleared from the site of injury. Most cells then become entrapped within the clot, which is sloughed during later phases of repair. The neutrophils remaining within viable tissue die in a few days and are phagocytized by the tissue macrophages or the modified wound fibroblasts. This marks the termination of the early inflammatory phase of repair. Although the neutrophils help create a favorable wound environment and serve as a source of pro-inflammatory cytokines, they are not essential to repair in uninfected wounds (Simpson and Ross, 2009).

The rapid increase in macrophage numbers under inflammatory conditions is predominantly caused by the emigration of monocytes from the vasculature, which then differentiates into macrophages to assist resident tissue macrophages at the wound site for a period lasting from days to weeks. In this manner, the responsive and adaptable pluripotent monocytes can differentiate into macrophages, whose functional properties are determined by the conditions they encounter at the site of mobilization. Like the neutrophils, the macrophages are phagocytes and thus carry out débridement and microbial killing. Unlike the neutrophils, the wound macrophages play a key role in the reparative phases of healing. Indeed, adherence to the ECM (which consists of a cross-linked supporting framework of collagen fibrils and elastin fibers, which is saturated with proteoglycans and other glycoproteins) stimulates monocytes to transform into phenotypes that have the ability to continually synthesize and express the various cytokines necessary for their survival, as well as for the initiation and propagation of new tissue formation in wounds. On arrival at the site of inflammation, macrophages participate in bacterial killing via mechanisms that parallel those of the neutrophils. Three inducible, secreted, neutral proteinases have been identified in macrophages: elastase, collagenase, and plasminogen activator (PA). These proteinases aid in degradation of damaged tissue and debris, which must be cleared before repair can proceed. Despite the new data gleaned from the study on mice without macrophages (Martinet al., 2003) acute inflammation is still considered crucial to the normal outcome of wound repair. Indeed, macrophages are regarded as the major inflammatory cell responsible not only for debridement but also for recruitment of other inflammatory and mesenchymal cells, and for subsequent induction of angiogenesis, fibroplasia, and epithelialization. Thus, a general approach for improving wound repair may be to recruit or possibly activate monocytes. For example, it has recently been shown that priming a planned incision site with recombinant pro-inflammatory cytokines nearly doubles the breaking strength of an acute wound (Smith et al., 2000). Likewise, honey and sugar applied to open wounds have been shown to enhance fibroplasia and epithelialization, possibly via their chemo attractant and stimulatory activity on the tissue macrophages (Molan, 1999) and (Swaim and Bohling, 2003). Extensive scarring or fibrosis of any organ may cause catastrophic loss of function of that organ. In the horse, a comparable condition is the development of exuberant granulation tissue in skin wounds. Wilmink and colleagues believe this is related to a deficient but protracted inflammatory response in the horse when compared with ponies, especially when wounds are located at the distal aspect of the limb. They found that the number of poly-morpho-nuclear leukocytes (PMNs) was high in ponies during the first 3 weeks after experimental full- thickness wounding, but it subsequently decreased rapidly, whereas in the horse the initial number of PMNs was lower, but it remained persistently elevated during the entire 6- week study (Wilmink et al., 1999). Furthermore, peripheral blood leukocytes from ponies produce more reactive oxygen species essential to bacterial killing than do those of horses (Wilmink et al., 2003), which corresponds to the more pronounced initial inflammatory response and to the better local defense against wound infection clinically apparent in the pony. A handful of equine studies have been undertaken with the intent of encouraging a powerful yet brief acute inflammatory response and thus limiting the subsequent fibrosis that appears in response to injury to the distal portion of the limb in horses. Wilson and colleagues found that although an activated macrophage supernate effectively restrained proliferation of equine fibroblasts in vitro, no significant in vivo effects were found on distal limb wounds. Another study found that a protein-free dialysate of calf blood (Solcoseryl, Solco Basle Ltd., Birsfelden, Switzerland) provoked a greater inflammatory response, with faster formation and contraction of granulation tissue within deep wounds (Wilmink et al., 2000). Subsequently, it inhibited repair by causing protracted inflammation and delaying epithelialization. Finally, a field study was recently performed to determine the efficacy of Vulketan gel (Janssen Animal Health, Beerse, Belgium) in preventing exuberant formation of granulation tissue in equine lower limb wounds (Engelen et al., 2004). The active ingredient appears to antagonize serotonin-induced suppression of wound macrophages, thus allowing a strong, effective inflammatory response to occur.

2) Cellular proliferation

Fibroplasia. The proliferative phase of repair comes about as inflammation subsides and is characterized by the eventual appearance of red, fleshy granulation tissue, which ultimately fills the defect. Although the earliest part of this phase is very active at the cellular level, this does not immediately translate into a gain in wound strength. Indeed, during the first 3 to 5 days after injury, mesenchymal cells such as fibroblasts and endothelial and epithelial cells are rapidly invading the wound in preparation for matrix synthesis and maturation; however, these latter reinforcing mechanisms lag somewhat. Granulation tissue is formed by three elements that move into the wound space simultaneously: macrophages debride and produce cytokines and growth factors, which stimulate angiogenesis and fibroplasia; fibroblasts proliferate and synthesize new ECM components; and new blood vessels carry oxygen and nutrients necessary for the metabolism and growth of mesenchymal cells, and confer to the granulation tissue (Singer and Clark, 1999). This stroma of which fibronectin and hyaluronan are major components, replaces the fibrin containing clot to provide a physical barrier to infection and, importantly, to proffer a surface across which mesenchymal cells can then migrate. A number of matrix molecules, as well as chemo attractants, cytokines, and growth factors released by inflammatory cells, are believed to stimulate fibroblasts from adjacent uninjured skin to proliferate and express integrin receptors to assist migration into the wound space. Integrins are trans membrane proteins that act as the major cell-surface receptors for ECM molecules and thus mediate interactions and transduce signals between cells and their environment. They are particularly critical to the migratory movements exhibited by wound-healing cells. Migration immediately precedes advancing capillary endothelial buds but follows macrophages, which have cleared a path by phagocytizing debris. Fibroblasts themselves also possess an active proteolytic system to aid migration into the cross-linked fibrin blood clot; proteinases include PA, various collagenases, gelatinase, and stromelysin. Once fibroblasts have arrived within the wound space, they proliferate and then switch their major function to protein synthesis and commence the gradual replacement of provisional matrix by a collagenous one, probably under the influence of various cytokines and growth factors. As the wound matures, there is a marked increase in the ratio of type I (mature) to type III (immature) collagen; proteoglycans also become abundant within the mature matrix. The greatest rate of connective tissue accumulation within the wound occurs 7 to 14 days after injury, and thus this is the period with most rapid gain in tensile strength. Thereafter, collagen content levels off as fibroblasts retract their synthetic machinery; this corresponds to a much slower gain in wound strength, which occurs as the wound remodels. The fibroblast-rich granulation tissue is then replaced by a relatively avascular and acellular scar as the capillary content regresses and fibroblasts either undergo apoptosis or acquire smooth-muscle characteristics and transform into myofibroblasts that participate in wound contraction. The latter phenomena are regulated by the physiologic needs and/or the micro-environmental stimuli present at the wound site. It appears that if the signal to down regulate fibroblast activity is delayed beyond a specific time point, apoptosis is permanently impaired, which ultimately leads to an imbalance between collagen synthesis and degradation and the formation of excessive scar tissue (Luo et al., 2001). Undeniably, repair of full-thickness wounds is subject to excessive formation of granulation tissue, with subsequent delays in epithelialization and contraction, especially when wounds are located at the distal aspect of the limb. Surprisingly, in vitro fibroblast growth from tissues isolated from the horse limb is significantly less rapid than growth of fibroblasts from the horse trunk (Bacon et al., 2000). In vivo, an elevated and persistent mitotic activity exists in distal metatarsal wounds of horses, compared with the activity present in wounds healing normally on the hindquarters (Leault et al., 2004). In a recent study in wounds of the distal limbs of horses, we found that the silicone dressing surpassed a conventional permeable, non-adherent dressing for preventing the formation of exuberant granulation tissue and improving tissue quality (Ducherme-Desjarlais et al., 2005)

Angiogenesis. Besides initiating the inflammatory response through interaction with leukocytes, microvascular endothelial cells play a key role in the proliferative phase of repair. The formation of new capillary blood vessels from preexisting ones (angiogenesis) is necessary to sustain the granulation tissue newly formed within the wound bed. Angiogenesis, in response to tissue injury and hypoxia, is a complex and dynamic process mediated by diverse soluble factors from both serum and the surrounding ECM environment in particular, angiogenic inducers including growth factors, chemokines, angiogenic enzymes, endothelial cell–specific receptors, and adhesion molecules (Liekenset al., 2003), many of which are released during the previous inflammatory phase of repair. Construction of a vascular network requires sequential steps that include augmented microvascular permeability, the release of proteinases from activated endothelial cells with subsequent local degradation of the basement membrane surrounding the existing vessel, migration and sprouting of endothelial cells into the interstitial space, endothelial cell proliferation and formation of granulation tissue, differentiation into mature blood vessels, and stabilization, eventually followed by regression and involution of the newly formed vasculature as the tissue remodels (Li et al., 2003). Angiogenic stimuli are down regulated or the local concentration of inhibitors increases and most of the recently formed capillary network quickly involutes through the activity of matrix metalloproteinases (MMPs) (Zhu et al., 2000) and apoptosis of endothelial cells. The wound color becomes correspondingly paler as the rich capillary bed disappears from the granulation tissue. Exuberant granulation tissue that develops in wounds of the lower limbs of horses is characterized

To cite this paper: Seid AM and Birhan M (2019). A Review on Equine Wound Management and Healing Process· *Online J. Anim. Feed Res.*, 9(2): 68-85. www.ojafr.ir

microscopically by a great number of microvessels. Although the reason angiogenesis is more prominent in this location remains obscure, it is tempting to speculate that the regional paucity of blood supply may impart an effect via up regulation of various angiogenic factors. Indeed, hypoxia is known to stimulate proliferation and synthetic activity of fibroblasts. In support of this hypothesis, we have recently shown that although a greater number of microvessels are microscopically apparent within the granulation tissue of limb wounds in equines, their lumens are occluded significantly more often than the lumens of microvessels within thoracic wounds, which may corroborate the existence of a hypoxic environment in wounds of the lower limb. Thus, via up regulation of various angiogenic factors, hypoxia may lead to excessive fibrosis. Alternatively, deficient apoptotic signals may lead to persistence of micro capillary endothelial cells and subsequent angiogenic activity.

Epithelialization. All body surfaces are covered by epithelium, which acts as a selective barrier to the environment. Epithelium provides the primary defense against hostile surroundings and is a major factor in maintaining internal homeostasis by limiting fluid and electrolyte loss. The outer region of skin, a multilayered stratified squamous epithelium (the epidermis), interfaces with the musculoskeletal framework by means of a connective tissue layer (the dermis) and a fibrofatty layer (the subcutis). Epidermis is attached to the dermis at the level of the basement membrane, a thin, glycoprotein-rich layer composed primarily of laminin and type IV collagen. This attachment is mediated by hemidesmosomes, which physically attach the basal cells of the epidermis to the underlying dermis, as well as by vertically oriented type VII collagen anchoring fibrils, which bind the cytoskeleton. Although epithelial migration commences 24 to 48 hours after wounding, the characteristic pink rim of new epithelium is not macroscopically visible until 4 to 6 days later, although this is variable because the rate of wound closure depends on the animal species as well as on the wound site, substrate, and size. For example, epithelialization is accelerated in a partial thickness wound, because migrating cells arise not only from the residual epithelium at the wound periphery but also from remaining epidermal appendages. Furthermore, the basement membrane is intact in this type of injury, precluding its lengthy regeneration. On the other hand, during second-intention healing of a full-thickness wound, epithelialization must await the formation of a bed of granulation tissue to proceed. Wounds in the flank area of a horse epithelialize at a rate of 0.2 mm per day, compared with a rate as slow as 0.09 mm per day for wounds in the distal portion of limbs. In preparation for migration; basal epidermal cells at the wound margin undergo phenotypic alterations that favor mobility and phagocytic activity. Additionally, various degradative enzymes necessary for the proteolysis of ECM components are up regulated within cells at the leading edge, facilitating ingestion of the clot and debris found along the migratory route. The migratory route is determined by the array of integrin receptors expressed on the surface of migrating epithelial cells, for various ECM proteins. Indeed, a fundamental reason why migrating epidermis dissects thefibrin eschar from wounds is that normal epithelial cells cannot interact with the fibrinogen and its derivatives found within the clot because they lack the appropriate integrin. Once the wound surface is covered by epithelial cells that contact one another, further migration from the margin of the wound inward is inhibited by the expression within the ECM of laminin, a major cell adhesion factor for epithelial cells. Although initial migration does not require an increase in cellular multiplication, epidermal cells at the wound margin do begin to proliferate 1 to 2 days after injury to replenish the migratory front. This corresponds histologically to epithelial hyperplasia, as cellular mitosis increases17-fold within 48 to 72 hours. The new cells leapfrog over those at the wound margin to adhere to the substratum, only to be replaced in turn by other cells coming from above and behind. The newly adherent monolayer subsequently restratifies in an attempt to restore the original multilayered epidermis. In full-thickness wounds healing by second intention, such as those commonly managed in equine practice, provisional matrix is eventually replaced by a mature basement membrane zone. Repairing epidermis reassembles its constituents from the margin of the wound inward, in a zipper like fashion. Epidermal cells then revert to a quiescent phenotype and become attached to this new basement membrane through hemidesmosomes and to the underlying dermis through type VII collagen fibrils. This particular aspect of epithelialization is time consuming, occurring long after total wound coverage is apparent, which may explain the continued fragility of neoepidermis for extended periods after macroscopically complete repair. This is particularly evident in large wounds of the limb, where epidermis at the center is often thin and easily traumatized. Wounds in horses commonly fail to epithelialize altogether. This occurs in two distinct types of wounds: those in which fibroplasia are excessive and those of an indolent nature. In the former, protruding granulation tissue may act as a physical impediment to epithelial migration and it may inhibit epithelial cell mitosis. The relative absence of epithelial cells could in turn lead to persistent synthesis of fibrogenic growth factors by fibroblasts (Lepoole and Boyce, 1999) and defective apoptosis signaling, thus establishing a vicious cycle culminating in proud flesh formation. Conversely, indolent wounds possess a granulation bed of deficient quantity and quality, thus hindering migratory efforts by epithelial cells. In this case, it is critical to encourage the formation of a healthy granulation bed. Although hydrogel dressings have been advocated for this purpose, a recent study is not supportive (Dart et al., 2002). In the case of limb wounds presenting delays in epithelialization but possessing a healthy bed of granulation tissue, the value of skin grafting is undisputed. Grafting exerts a significant inhibitory effect on both endothelial cell and fibroblast growth

while enhancing proliferation and migration of epithelial cells. It is, however, critical that the graft be obtained from a site that normally heals well and in which contraction is a prominent feature (e.g., from the lateral cervical, abdominal, or pectoral regions). The inhibitory effect of grafts on fibroblast proliferation and collagen synthesis may be regulated by a soluble epithelial cell–derived product (Lepoole and Boyce, 1999) possibly a cytokine or a growth factor such as epidermal growth factor (EGF), which enhances epithelialization via positive effects on epithelial cell migration, proliferation, and differentiation (Burling et al., 2000). To encourage ingrowth of mesenchymal cells in indolent wounds during the proliferative phase, biomaterials such as collagen membranes and sponges have been developed and are appraised as improving rate and quality of repair. Collagen may function as a substrate for hemostasis; as a template for cellular attachment, migration, and proliferation; and as a scaffold for more rapid transition to mature collagen. A porous bovine collagen membrane was shown to generate a strong inflammatory response in full-thickness limb wounds of equines, which may augment the cytokine or growth factor content of wound tissues, although it did not significantly alter the total wound, or the epithelialization or contraction process. A commercially available collagen matrix derived from porcine small intestinal submucosa (Vet BioSISt, Cook Veterinary Products, Inc, Spencer, Ind.) and containing a plethora of proteins and growth factors, has been designed as a scaffold for tissue ingrowth and is promoted as reducing scarring. Regrettably, a recent study determined that it offers no apparent advantage over a nonbiologic, nonadherent synthetic dressing for treatment of small, granulating wounds of the distal limb of horses (Gomez et al., 2004)

Matrix Synthesis and Remodeling. In addition to epithelialization, contraction contributes to the successful closure of full-thickness wounds. Contraction is defined as a process whereby both dermis and epidermis bordering a full-thickness skin deficit are drawn from all sides centripetally over the exposed wound bed. Wound contraction is divided into three phases. An initial lag phase (wherein skin edges retract and the wound area increases temporarily for 5 to 10 days) occurs because significant fibroblastic invasion into the wound is a prerequisite for contraction. Subsequently, a period of rapid contraction is followed by a period of slow contractionas the wound approaches complete closure. The number of myofibroblasts found in a wound appears to be proportional to the need for contraction; thus, as repair progresses and the rate of contraction slows, this number decreases. During wound contraction, the surrounding skin stretches by intossusceptive growth, and the wound takes on a stellate appearance. Contraction ceases in response to one of three events: the wound edges meet and contact inhibition halts the processes of both epithelialization and contraction; tension in the surrounding skin becomes equal to or greater than the contractile force of the myofibroblasts; or, in the case of chronic wounds, a low myofibroblast count in the granulation tissue may result in failure of wound contraction despite laxity in the surrounding skin. In this case, the granulation tissue is pale and consists primarily of collagen and ground substance. Wound contraction is greater in regions of the body with loose skin than in regions where skin is under tension, such as the distal aspect of the limb. Although it has been speculated that the shape of the wound may influence the process of contraction, this does not appear relevant in wounds at the distal extremities where skin is tightly stretched and not easily moved. Skin grafts have been reported to inhibit contraction by preventing formation of myofibroblasts or by accelerating the myofibroblast life cycle. As contraction concludes, myofibroblasts disappear, either by reverting to a quiescent fibroblast phenotype or by apoptosis, primarily in response to reduced tension within the ECM (Grinell et al., 1999). The myofibroblast persists in fibrotic lesions, where it may be involved in further ECM accumulation and pathologic contracture but leading to significant morbidity particularly when it involves joints or body orifices. Significant differences exist with regard to contraction between horses and ponies and between distinct areas of the body. Wound contraction is clearly more pronounced in ponies than in horses (Wilmink et al., 1999) and the rate of contraction of limb wounds is at best 25% that of flank wounds. The conversion of ECM from granulation to scar tissue constitutes the final phase of wound repair and consists of connective tissue synthesis, lysis, and remodeling, also referred to as maturation. Collagen macromolecules provide the wound tissue with tensile strength as their deposition peaks within the first week in primary wound repair, and between 7 and 14 days in second-intention healing. Although this corresponds to the period of most rapid gain in strength, only 20% of the final strength of the wound is achieved in the first 3 weeks of repair. At this time, collagen synthesis is balanced by collagenolysis, which normally prevents accretion of excessive amounts of collagen and formation of pathologic scars. It appears that during the development of exuberant granulation tissue in horses, collagen synthesis continues unabated (Schwartz et al., 2002). The balance between synthesis and degradation determines the overall strength of a healing wound at a particular time. The first newly deposited collagen tends to be oriented randomly and therefore provides little tensile strength, whereas during remodeling the fibers re-form along lines of stress and therefore resist dehiscence more effectively. Crosslinking in the later formed collagen is also more effective, although never to the same extent as in the original tissue. A recent study has shown that newly accumulated collagen fibrils are disorganized in wounds at the distal aspect of the forelimb of horses but more normally organized in thoracic wounds (Schwartz et al., 2002).

FACTORS INFLUENCING WOUD HEALING

1) Patient Factors

Age and Physical Status: The patient's age and physical status may influence the rate of wound healing. They consequently also appear more susceptible to infection (Stotts and Wipke-Tevis, 2001). Although there currently is no similar data for the horse, in the author's experience it appears that young horses heal more readily and with fewer complications than do older horses.As Theoret points out, since horses, compared to companion animals, are less commonly affected by these diseases, they are generally not a concern (Theoret, 2006). An exception may be horses suffering from Cushing's disease (Pars intermedia dysfunction) in which high endogenous cortisol can suppress inflammation sufficiently to delay healing (Knottenbelt, 2007). Also, sincehigh concentrations of glucocorticoids are known to be immunosuppressive, it is logical that horses suffering from this malady may be more susceptible to wound infection.

Anemia/Blood Supply/Oxygen Tension: Because most of the oxygen in blood is carried by hemoglobin, it is intuitive that anemia should be an important factor in reduced oxygen delivery and impaired wound healing; this concept however, is unfounded. Data suggest that normovolemic anemia with a packed cell volume (PCV) >20, unrelated to malnutrition, cancer, or chronic infection, doesnot appear to affect wound healing. However, hypovolemia associated with hemorrhage and anemia or shock can greatly impair healing if not corrected (Huntet al., 2000). Decreased perfusion of the wound appears to be the cause of altered healing. Local tissue hypoxia that results from insufficient blood volume in hypovolemic patients inhibits many of the responses that initiate healing. An oxygen gradient exists between the nearest functioning capillary andthe wound edge. The oxygen tension near a wound capillary is between 60 and 90 mm Hg; however, near the advancing edge of granulation tissue the oxygen tension approaches 0 mm Hg. This decrease is caused by the diffusion gradient and the consumption of oxygen by cells at the wound margin. Since the activities of the new fibroblasts (migration, proliferation, and protein synthesis) rely on the rate at which new capillaries are formed, the wound tensile strength is limited by perfusion and tissue oxygen tension.The maturity and fragility of the new blood vessels forming in an acute wound appear to be affected by oxygen tension. New vessels forming in a hypoxic environment (13% inspired oxygen) are immature and bleed easily. Conversely, new vessels forming in a hyperoxic environment (50% inspired oxygen) are mature and form at a more rapid rate than do vessels in either a normoxic (21% inspired oxygen) or hypoxic environment (Hopf and Rollins, 2007). Reduced oxygen tension, besides inhibiting fi broblastic replication and migration, development of collagen, and tensile strength, also renders the wound more susceptible to infection by altering cellular phagocytic mechanisms (Hunt et al., 2000). When leukocytes ingest organisms and wound debris, more oxygen is consumed. Lack of sufficient oxygen slows the activity of leukocytes and decreases superoxide release, making the wound more susceptibleto infection (Hopf and Rollins, 2007). So correction of hypovolemia, and possibly the use of hyperbaric oxygen (HBO) therapy, should reduce the incidence of wound infection and allow healing to progress normally.

Malnutrition and Protein Deficiency. Wound healing is impaired with mild to moderate short or long-term protein energy malnutrition. It appears that the direction of patient is moving toward metabolically (positive or negative) at the time of injury or surgery is most important, since the adverse effect of protein energy malnutrition occurs well in advance of the external evidence of weight loss. Impaired nutrition can alter growth factor synthesis and fibroblastic proliferation, and limit hydroxy proline and collagen deposition, as well as impair immune functions and oxygen transport in healing patients. Insufficient lipid levels, important to inflammation and membrane stabilization, have also been shown to adversely affect wound healing (Stotts et al., 2001). The impairment in wound healing is easily reversed by providing adequate nutrition. Although this has not been proven in horses, it seems logical that there would be an effect of inadequate nutrition on wound healing in this species as well. Hypoproteinemia alone adversely affects wound healing primarily by altering fibroplasia, angiogenesis, remodeling, and gain in tensile strength, consequently prolonging the repair phase of healing. The impairment in wound healing from hypoproteinemia is seen well in advance of alterations in the plasma protein levels. As an example, albumin fractions are depleted almost immediately following withdrawal of protein from the diet. Since albumin is the major oncotic pressure stabilizer in the intravascular compartment, it is not surprising that a decreased serum concentration is associated with poor healing outcomes (Stotts et al., 2001). Even though the alteration in healing is not strongly correlated to plasma protein levels, when these levels fall to 6 g/dl, healing is retarded. Below 5.5 g/dl, a 70% incidence of wound disruption is expected, and below 2 g/dl, wound healing is disrupted, edema occurs, and death ensues. Because fats can be synthesized from carbohydrates and carbohydrates can be synthesized from protein but protein can only be produced from protein or its digested byproducts (amino acids and peptides) a protein rich diet is required to counteract the adverse effects on wound healing. Feeding D-L methionine to protein-deficient animals reverses the retardation in wound healing (Plumb, 2005).

To cite this paper: Seid AM and Birhan M (2019). A Review on Equine Wound Management and Healing Process· *Online J. Anim. Feed Res.*, 9(2): 68-85. www.ojafr.ir

Dehydration: Dehydration of the patient as well as the wound can negatively affect wound healing. The poor perfusion of peripheral tissues in the dehydrated patient is thought to be the reason that healing is delayed in these subjects (Peacock, 1984). This problem can easily be rectified by hydrating the patient. Wound dehydration will be discussed laterunder "nature of the wound."

2) Wound Factors

Trauma. Excessive trauma, associated with the wound or at a site or sites remote from the wound (e.g., multiple lacerations or multiple fractures) can negatively affect repair and make the wound more susceptible to infection. When the effects of simultaneous trauma from either fracture or muscle contusion elsewhere on the body were examined, a delay in wound tensile strength was observed out to 15 days post-trauma. A delay in gain of wound tensile strength was also observed at the other sites when a second wound was made within 14 days of the first, and the degree and loss in gain of wound tensile strength was proportional to the severity of trauma (Peacock, 1984).

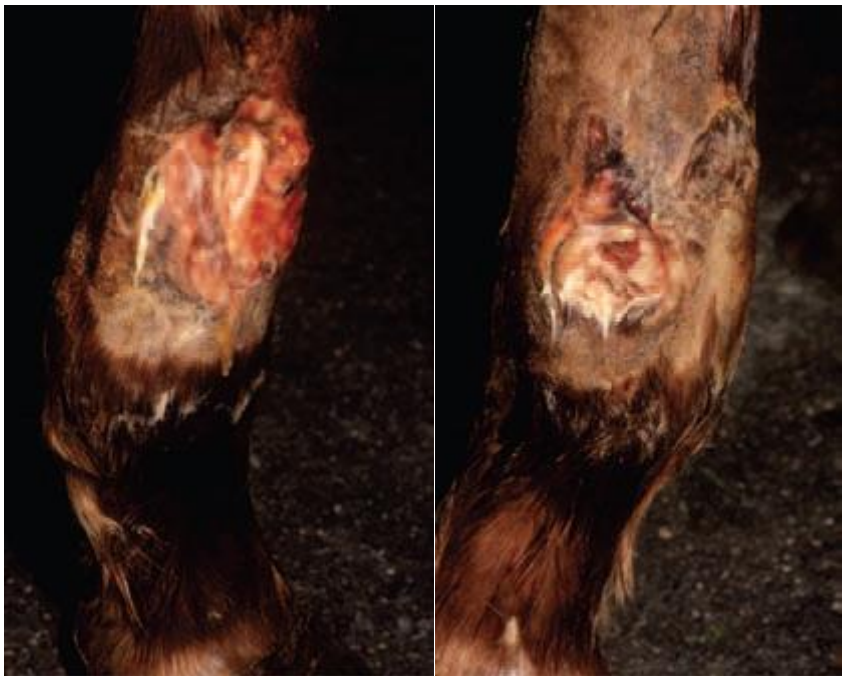


Figure 2. Excessive trauma to the distal metacarpal region

Age of Wound: A chronic wound (slow or non-healing) is often associated with an underlying problem that has exerted a negative impact on the normal progress in repair. Chronic inflammation from foreign bodies, necrotic tissue, repetitive mechanical trauma, and the application of caustic agents is a common cause. The goal in treating a chronic wound is to eliminate the causal agent/agents and convert the wound environment to one that closely resembles that of the acute wound; this is best done by wound debridement. In a case in which repeated mechanical trauma is the underlying cause, the use of appropriate bandaging/splinting techniques to immobilize the region is most important to ensure a successful outcome (Kane, 2001).

Location: Wounds of the distal extremities (carpus/tarsus and below) of horses heal more slowly and are more problematic than wounds of the upper body and head regions. Specifically, delays in epithelialization and contraction, as well as the propensity to develop exuberant granulation tissue (EGT), commonly afflict full-thickness wounds of the distal limbs (Theoret et al., 2001). Although the causes of problematic healing in the horse's distal limbs have yet to be completely clarified, several have been proposed. Better blood supply, a greater amount of adnexal structures, and the thinner epidermis covering the head and neck contribute to the more rapid and cosmetic repair occurring in these regions. Wounds of the distal extremities have an absence of underlying musculature, may be near highly mobile joints and bony prominences, and are often more contaminated than are body wounds (Cochrane et al., 2003). Differences between growth characteristics of trunk and limb fibroblasts and a tardy and prolonged inflammatory response are believed to contribute to the development of EGT, especially in limb wounds, in the horse. A study showed that fibroblasts isolated from the horse distal limb grow significantly more slowly than those of the trunk. The horse displays a deficient and protracted inflammatory response compared to the pony, especially in wounds involving the distal extremities (Wilmink, 1999). Furthermore, leukocytes from horses appear to be poorly equipped to kill bacteria compared to those of ponies (Wilmink, 2003). Moreover, their local cytokine profile is skewed in favor of fibrogenic mediators (Schwartz et al., 2002). Deficient contraction of limb wounds compared to body wounds may be due to a poor arrangement of myofibroblasts, precluding an ordered contractile activity.

To cite this paper: Seid AM and Birhan M (2019). A Review on Equine Wound Management and Healing Process· *Online J. Anim. Feed Res.*, 9(2): 68-85. www.ojafr.ir

Cytokine profiles may also negatively affect contractility (Wilmink, 2001). Wounds subjected to excessive movement, such as those located over highly moveable joints or those oriented perpendicular to lines of skin tension (e.g., perpendicular to the limb's long axis), are often slow to heal, and they usually form a disproportionate amount of scar tissue. Movement can also occur between healing skin and underlying tendon or muscle or between the heel bulbs, causing the wound edges to gape during weight bearing (Knottenbelt, 1997).

Nature of the Wound

A) Type: Degloving injuries that damage (strip off) the periosteum and the paratendon are more susceptible to infection and subsequent osteomyelitis or septic tendonitis because of loss of blood supply. In these cases, soft tissue coverage of the site should be achieved as soon as possible because of the increased risk of bony sequestration, tendon degeneration, and uncontrollable bone and soft tissue infection if the blood supply is not quickly re-established. Wounds in which a flap of tissue is at odds with the distribution of blood vessels in the extremities often experience delays in healing and are more susceptible to infection. In most cases it is best to delay suturing these wounds until a healthy bed of granulation tissue forms; this will ensure a good blood supply to support the healing of the skin flap following suturing. Stabilizing the flap into a somewhat normal position using a few large sutures, while awaiting definitive treatment, will prevent skin flap retraction (Stashak, 2003).

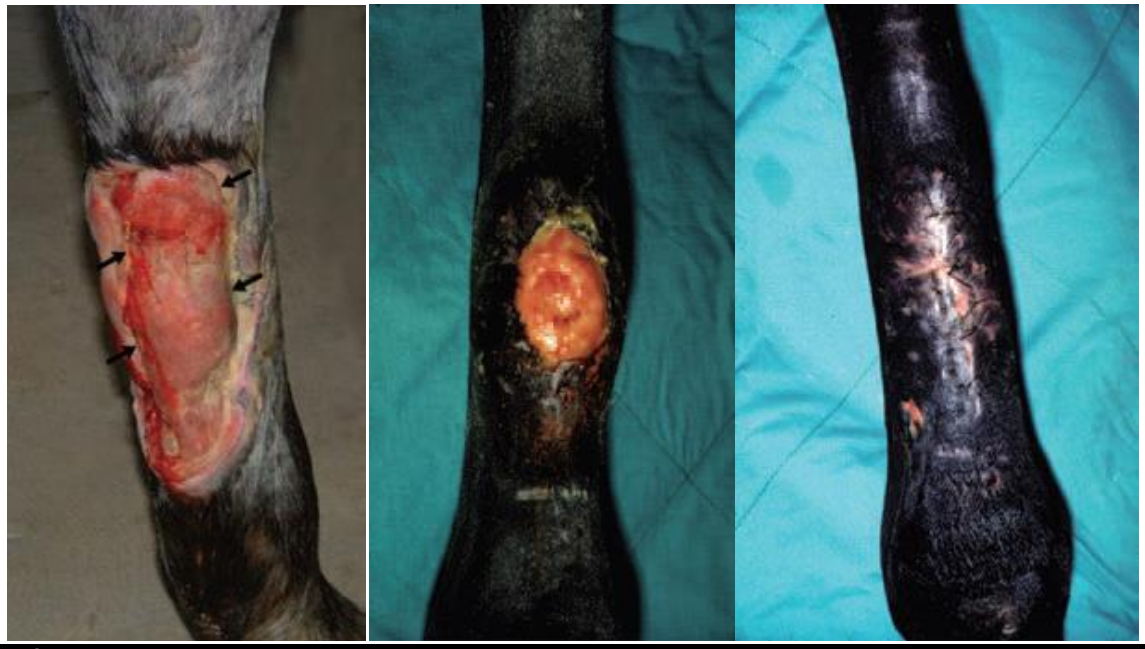


Figure 3. Wounds in which movement between the healing skin and underlying tendon will either delay or prevent healing.

B) Degree of Contamination. Wounds may be classified according to contamination and increasing risk of infection. A clean wound is one created surgically under aseptic conditions in situations where a contaminated site is not entered. Wounds are considered clean, clean contaminated, contaminated, or dirty contaminated/infected. As would be expected, the greater the contamination the greater the risk for infection. Dirty wounds have a 25-fold greater infection rate than do clean wounds. Wounds contaminated with fecal material and dirt run a high risk of infection despite therapeutic intervention; indeed, feces may contain up to 1011 microorganisms per gram (Stashak, 2006).Specific infection potentiating fractions (IPFs) found in the organic and inorganic components of soil increase the wound's susceptible to infection. These highly charged fractions reduce the effects of white blood cells, decrease humoral factors, and neutralize antibodies. Consequently, as few as 100 microorganisms can cause infection in wounds contaminated with soil. The metabolic impact of contaminating bacteria (bioburden) on tissues can significantly impair healing (Robson, 1997).

C) Wound Fluids. Wounds with pockets that allow exudate or seroma/hematoma to accumulate are often slower to heal and are susceptible to infection. While persistent contact with inflammatory products present in the exudate during the repair phase is believed to cause the delay in repair, accumulation of seroma/hematoma further provides an excellent medium for bacterial growth, thus making the wound more susceptible to infection. Expanding fluid pressure from the exudate/seroma/hematoma may also be great enough to alter the local blood supply (Stashak, 2003). Drainage of the fluid-filled pocket followed by bandaging, where applicable, is usually all that is needed. Experiments have identified differences between acute and chronic wound fluids in humans (Rao et al., 1995). Metalloproteinases, essential in the various phases of healing but detrimental in the case of persistent up-regulation, were found to be 5 to 10 times higher in chronic wound fluid. Serine proteinases that degrade fibronectin and impede

To cite this paper: Seid AM and Birhan M (2019). A Review on Equine Wound Management and Healing Process. *Online J. Anim. Feed Res.*, 9(2): 68-85. www.ojafr.ir

collagen synthesis and epithelialization were also found to be increased in chronic wound fluids. Interestingly, when a chronic wound reverts to active healing, the levels of metalloproteinase activity decrease significantly, which parallels the processes observed in normally healing acute wounds (Trengrove et al., 1999).

D) Infection: Infection is considered to be a major cause of delayed wound healing, reduced gain of tissue tensile strength, as well as dehiscence following wound closure. Potential pathogenic bacteria bind to extracellular matrix proteins (e.g., fibronectin), which may limit the latter's availability for promoting migration of mesenchymal cells and consequently bear a direct negative effect on wound healing. Additionally, bacteria that produce exotoxins (e.g., Clostridium spp, S. pyogenes, S. aureus) cause more tissue damage, creating a microenvironment conducive to their survival. Those with thick capsules (e.g., S. pyogenes, S. aureus, and Klebsiella pneumoniae) are more resistant to phagocytosis by leukocytes. During the process of bacterial degradation, released endotoxins can activate coagulation pathways which may cause thrombosis of the microvasculature or systemic organ or immune dysfunction, and activate macrophages to release more inflammatory mediators (Brumbaugh, 2005).

CONCLUSION AND RECOMMENDATION

A vital trait of living organisms continually subjected to insults from the environment is their capacity for self-repairs. Whether the injury is a deliberate act of surgery or accidental, it generates an attempt by the host to restore tissue continuity. The two processes involved in wound healing are repair and regeneration. Regeneration entails the replacement of damaged tissue with normal cells of the type lost, and this is possible only in tissues with a sustained population of cells capable of mitosis, such as epithelium, bone, and liver. Repair is a stopgap reaction designed to reestablish the continuity of interrupted tissues (Theoret, 2005). The combination of skin loss and the amount of redundant or loose skin surrounding a wound has an obvious influence on the clinician's ability to close a wound and the extent to which wound contraction will contribute to the final result. The effects of the location of the wound on the body as well as breed differences on wound healing in horses are well recognized (Jacobs, 1984) as is the influence of surrounding skin tension on wound contraction (Walton, 1972). A large retrospective study revealed that primary closure was successful in only 24% of horse wounds and 39% of pony wounds, more than half of which were located on the limb. Thus, a significant number of wounds must heal by second intention. Unfortunately, this type of repair leads to formation of a much larger scar tissue than that formed after successful primary closure, and function and appearance may be adversely affected. Wound healing is part of normal body maintenance and depends on the patient's general state of health, blood supply/anemia/local oxygen gradient, location, vitamins (A, E, and C) and minerals (Zinc).

From this review the following recommendations are forwarded:

- Financial and time constraints are often imposed on the practitioner, so it is advisable to spend time discussing the owner's expectations, the possible complications, and the costs associated with these procedures before beginning wound management.
- The extent to which some reconstructive and skin mobilization techniques can be used depends on the presence of adequate tissue surrounding the wound.
- The practitioner should consider location, nature, cleanness of wound before starting management.
- Animals should offered balanced nutrition in adequate amounts prior to surgery and/or elective surgery.
- Therefore, additional investigation should conduct to find out a cost effective management of wound with locally available materials.

DECLARATIONS

Corresponding author:
Email: addisvet838@gmail.com

Acknowledgment
The authors' heartfelt thanks will also go to the University of the Gondar for resource supporting.

Authors' contributions
MB conceived the review, coordinated the overall activity, and reviewed the manuscript. AM supervising all in all activities

Availability of data and materials
Data will be made available upon request of the primary author

Consent to publish
Not applicable.

Competing interests
The authors declare that they have no competing interests.

REFERENCES

Angel DE, Morey P, Storer JG & Mwipatayi BP (2008): The great debate over iodine in wound care continues; A Review of the Literature, Wound Practice & Research, 16(1): 6-21.

Aramwit P, Muangman P, Namviriyachote N & Srichana T (2010): *In vitro*evaluation of the antimicrobial effectiveness and moisture bindingproperties of wound dressings, Int J Mol Sci. 11(8): 2864–74.

Asharani PV, Hande MP & Valiyaveettil S (2009): Anti-proliferative activity of silver nanoparticles, BMC Cell Biol. 10: 65.

Auer J and John Stick J (2006): Equine Surgery (third edition).

Ayello EA, Baranoski S, Cuddigan J, Sibbald RG. (2008): Wound Debridement and Care Essentials; Practice Principles, In. Baranoski, S., Ayello, EA., eds. 2nd Edition, Lippincott Williams & Wilkins, Ambler, PA.

Bacon Miller C, Wilson DA, Keegan KG (2000): Growth characteristics of fibroblasts isolated from the trunk and distal aspect of the limb of horses and ponies, Vet Surg.29:1.

Bailey JV (2003): Repair of large skin defects on the limbs of horses, Wien Tierarztl Mschr 1991; 78:277.

Biazar E, Roveimiab Z, Shahhossein iG, Khataminezhad M, Zafari M & Majdi A (2012): Biocompatibility evaluation of a new hydrogel dressing based on polyvinylpyrrolidone/polyethylene glycol. J Biomed Biotechnol.

Boateng, JS, Matthews KH, Stevens HN & Eccleston GM (2008): Wound healing dressings and drug delivery systems; A Review. J Pharm Sci Aug. 97(8):2892–923.

Burling K, Seguin MA, Marsh P (2000): Effect of topical administration of epidermal growth factor on healing of corneal epithelial defects in horses; Am J Vet Res.61:1150. 259

Chaikof EL, Matthew H, Kohn J, Mikos AG, Prestwich GD & Yip CM (2002): Biomaterials and scaffolds in reparative medicine. Ann N Y Acad Sci, Jun. 961:96–105.

Chaudhry HR, Bukiet B, Siegel M (1998): Optimal patterns for suturing wounds, J Biomech.31:653

Cochrane CA (1997): Models in vivo of wound healing in the horse and the role of growth factors. Vet Dermatol.8.

Cochrane CA, Pain R, Knottenbelt DC: In vitro wound contraction in the horse: differences between body and limb wounds. Wounds 2003; 15:175

Collins MN, Friend TH, Jousan FD, Chen SC (2000): Effect of density on displacement, falls, injuries, and orientation during horse transportation ; *Appl Anim Behav Sci*.67(3):169–179.

Cooper RA. (2007). Iodine revisited. Int Wound J 2007 Jun; 4(2):124–37.

Cooper R (2004): A Review of the Evidence for the Use of Topical Antimicrobial Agents in Wound Care; World Wide Wounds.

Cooper R (2008): Using honey to inhibit wound pathogens. Nurs Times Jan 22–28; 104(3):46,89.79.

DACA (Drug Administration and control Authority) (2006): Standard treatment Guideline for veterinary practice of Ethiopia; Pp: 209-211.

Dart AJ, Cries L, Jeffcott LB (2002): Effects of 25% propylene glycol hydrogel (Solugel) on second intention wound healing in horses, Vet Surg; 31:309.

Davies P and Rippon M (2010): Comparison of foam and hydrocolloid dressings in the management of wounds; A Review of the Published Literature. World Wide Wounds [Online].

Deeth M, Oldfield A, Hampton S and Goodwin L (2012): Rapid Action Capillary Dressings. In: Medical A (ed), Advancis Medical.

Dinka H, Shelima B, Abalti A, Geleta T, Mume T (2006): Socio-economic importance and management of carthorses in the mid rift valley of Ethiopia. Proceedings of the 5th International Colloquium on Working Equines.

Dressel C, Fürst A, Imhof A (2004): Einsatz von Small Intestine Submucosa (SIS) zur Wiundversorgung bei 11 Pferden: Erste Erfahrungen. Wien Tieraerztl Mschr, 91:142.

Ducharme-Desjarlais M, Céleste CJ, Lepault É (2005): Effect of a silicone-containing dressing on exuberant granulation tissue formation and wound repair in the horse, Am J Vet Res [in press].

Dumville JC, Deshpande S, O'Meara S and Speak K (2011): Foam dressings for healing diabetic foot ulcers.

Dumville JC, O'Meara S, Deshpande S and Speak K (2011): Hydrogel dressings for healing diabetic foot ulcers.

Dutta J, Tripathi S and Dutta PK (2011): Progress in antimicrobial activities of chitin, chitosan and its oligosaccharides; A Systematic Study Needs for Food Applications. Food Science and Technology International, 18(1): 3–34.

Eaglstein WH (2001). Moist wound healing with occlusive dressings: a clinical focus. Dermatol Surg 2001 Feb; 27(2):175–81.

Falanga V, and Harding K (2002): The Clinical Relevance of Wound Bed Preparation.

FAOSTAT (2012): Food and Agricultural Statistical Database: http://www.fao.org/corp/statistics/ access online/.

Flores A & Kingsley A (2007): Topical Antimicrobial Dressings; An Overview. Wound Essentials, 2:182–5.

Gomez JH, Schumacher J, Lauten SD (2004): Effects of 3 biologic dressings on healing of cutaneous wounds on the limbs of horses, Can J Vet Res, 68:49.

Goodrich LR, Moll HD, Crisman MV (2000): Comparison of equine amnion and a nonadherent wound dressing material for bandaging pinch-grafted wounds in ponies, Am J Vet Res, 61:326.

Griffiths DA, Simpson RA, Shorey BA, Speller DC, Williams NB (2003): Single-dose peroperative antibiotic prophylaxis in gastrointestinal surgery. *Lancet*.2(7981):325–328.

Gusman D (1995): Wound closure and special suture techniques, J Am Podiatr Med Assoc, 85:2.

Harper, Michael S, Berkeley CA (2001): Debridement, Paradigm Press.

Haydock DA, Graham LH (1986): Impaired wound healing in surgical patients with varying degrees of malnutrition. J Parenterol Enteral Nutr,10:550

Hernandez J, Hawkins DL (2001): Training failure among yearling horses. *Res Am J Vet*, 62(9):1418–1422.

Heughan, C., Grislis, G., Hunt, T. (1974): The effect of anemia on wound healing. Ann of Surg, 179:163

Hollander, JE., Richman, PB., Werblud, M. (1998) : Irrigation in facial and scalp lacerations; Does it alter outcome? Ann Emerg Med, 31:73.

Hopf, HW., Rollins, MD. (2007): Wounds; An Overview of the Role of Oxygen. Antioxid Redox Signal, 9:1183

Hunt, TK., Hopf, W., Hussain, Z. (2006): Physiology of wound healing. Skin Wound Care, 13:6.

Jacobs, KA., Leach, DH., Fretz, PB. (1984): Comparative aspects of the healing of excisional wounds on the leg and body of horses, Vet Surg, 13:83.

Jones, V., Grey, JE.and Harding, KG. (2006): Wound dressings. BMJ. Adv Apr 1, 332(7544):777–80.

Jonsson, K., Jensen, JA., Goodson, WH. (1987): Assessment of perfusion in postoperative patients using tissue oxygen measurements. Br J Surg, 74:263.

Jude, EB., Apelqvist, J., Spraul, M. and Martini, J. (2007): Prospective randomized controlled study of Hydrofiber dressing containing ionic silver or calcium alginate dressings in non-ischaemic diabetic foot ulcers. Diabet Med Mar, 24(3):280–8.

Juris, S., Mueller, A., Smith, B., Johnston, S., Walker, R. and Kross, R. (2011): Biodegradable Polysaccharide Gels for Skin Scaffolds. J Biomater Nanobiotechnol, 2(3):216–25.

Kane, DP. (2001): Chronic wound healing and chronic wound management. Chronic wound care; A Clinical Source Book for Healthcare Professionals. *3rd ed*. Wayne, PA: HMP Communications, p.7.

Kim, TH., Kim, M., Park, HS., Shin, US., Gong, MS. and Kim, HW. (2012): Size-dependent cellular toxicity of silver nanoparticles. J Biomed Mater Res A Apr, 100(4):1033–43.

Knottenbelt, DC. (1997) : Equine wound management; Are there significant differences in healing at different sites on the body? Vet Dermatol, 8:273

Knottenbelt, DC., Pascoe, RR. (1994): Color Atlas of Diseases and Disorders of the Horse.

Kwakman, PH. and Zaat, SA. (2012): Antibacterial components of honey. IUBMB Life Jan, 64(1):48–55.

Lepault, É., Céleste, CJ., Doré, M. (2004): Comparative study on microvascular occlusion and apoptosis in normal and pathologic wounds in the horse, Wound Repair Regen [submitted].

LePoole, IC., Boyce, ST. (1999): Keratinocytes suppress TGF-□1 expression by fibroblasts in cultured skin substitutes, Br J Dermatol, 140:409.

Li, J., Zhang, YP., Kirsner, RS. (2003): Angiogenesis in wound repair: Angiogenic growth factors and the extracellular matrix, Microsc Res Tech, 60:107.

Liekens, S., De Clerq, E., Neyts, J. (2001): Angiogenesis; Regulators and clinical applications, Biochem Pharmacol, 61:253.

Losi, P., Briganti, E., Costa, M., Sanguinetti, E. and Soldani, G. (2012): Silicone-coated nonwoven polyester dressing enhances re-epithelialisation in a sheep model of dermal wounds. J Mater Sci; Mater Med, 23:2235–2243.

Luo, S., Benathan, M., Raffoul, W. (2001): Abnormal balance between proliferation and apoptotic cell death in fibroblasts derived from keloid lesions. Plast Reconstr Surg, 107:87.

Macava, D. and Spector, M. (2012): Injectable hydrogel materials for spinal cord regeneration: a review. Biomed Mater (Bristol, UK), 7(012001).

Martin, P., D'Souza, D., Martin, J. (2003): Wound healing in the PU.1 null mouse: Tissue repair is not dependent on inflammatory cells, Curr Biol, 13:1122.

McEwan, C. (2000): Wound cleansing and dressing, Am J Clin Dermatol, 1:57.

Mekuria, S., Matusala M. and Rahameto, A. (2013): Management practices and welfare problems encountered on working equids in Hawassa town, Southern Ethiopia; Journal of Veterinary Medicine and Animal Health, 5(9): 243-250.

Mengistu, A. (2003): The genetic resources perspective of equines in Ethiopia and their contribution to the rural livelihoods. Proceedings of the 11th Annual Conference of the Ethiopian Society of Animal Production (ESAP). Addis Ababa, Ethiopia, 81-85.

Molan, PC. (1999): The role of honey in the management of wounds, J Wound Care, 8:415.

Molan, PC. (2006): The Evidence Supporting the Use of Honey as a Wound Dressing. Int J Low Extrem Wounds, 5(1):40–54.

Moscati, R., Mayrose, J., Fincher, L. (1998): Comparison of normal saline with tap water for wound irrigation, Am J Emerg Med, 16:379.

Moy, LS. (1993): Management of acute wounds. Dermatol Clin, 11:759

Myers, BA. (2008): Wound management principles and practice. 2nd ed. Upper Saddle River, NJ: Pearson.

Ohtani, T., Mizuashi, M., Ito, Y. and Aiba, S. (2007): Cadexomer as well as cadexomer iodine induces the production of proinflammatory cytokines and vascular endothelial growth factor by human macrophages; Exp Dermatol Apr, 16(4):318–23.

Ovington, LG. (2001): Battling bacteria in wound care. Home Health Nurse, 19:622.

Peacock, EE. (1984): Collagenolysis and the biochemistry of wound healing. In E Peacock ed. *Wound repair (3rd edition)*. Philadelphia: WB Saunders, p.102

Purna Sai, K. and Babu, M. (2000): Collagen based dressings; A review, Burns, 26:54.

Queen, D., Orsted, H., Sanada, H. and Sussman, G. (2004): A dressing history. Int Wound J, 1(1):59–77.

Rao, CN., Ladin, DA., Liu, YY., Chilikuri, K. (1995): Alpha 1-antitrypsin is degraded and non-functional in chronic wounds but intact and functional in acute wounds; the inhibitor protects fbronectin from degradation by chronic wound fluid enzymes. J Invest Dermatol, 105:572

Robson, MC. (1997): Wound infection; A failure of wound healing caused by an imbalance of bacteria. Surg Clin North Am, 77:637

Rodeheaver, GT. (2001): Wound cleansing, wound irrigation, wound disinfection. In Kraner D, Rodeheaver GT, Sibbald RG, editors: Chronic Wound Care: A Clinical Source Book for Healthcare Professionals, ed 3, Wayne, Pa, HM Communications.

Schwartz, AJ., Wilson, DA., Keegan, KG. (2002): Factors regulating collagen synthesis and degradation during second intention healing of wounds in the thoracic region and the distal aspect of the forelimb of horses; Am J Vet Res, 63:1564

Sells, P., Pinchbeck,G., Mezzane, H., Ibourki, J. and Crane, M. (2010): Pack wounds of donkeys and mules in the Northern High Atlas and lowlands of Morocco. Equine Veterinary Journal, 42(3): 219-226.

Sevendsen, E., (2008): The professional handbook of the donkey; 4th ed. London, Whittet Books Limited.

Singer, AJ. and Clark, RAF.(1999): Cutaneous wound healing; N Engl J Med,341:738-746.

Singer, ER., Saxby, F., French, NP. (2003): A retrospective case-control study of horse falls in the sport of horse trials and three-day even ting. *Equine Vet J*., 35(2):139–145.

Smith, PD., Kuhn, MA., Franz, MG. (2000): Initiating the inflammatory phase of incisional healing prior to tissue injury, J Surg Res, 92:11.

Stashak, TS. (1991): Selected factors that affect wound healing. In T Stashak ed. *Equine wound management (1st edition)*. Philadelphia; Lea and Febiger.

Stashak, TS. (2003): Current concepts in wound management in horses; parts I-III. Proc North Am Vet Conf, 17:231

Stashak, TS. (2006): Wound Infection: contributing factors and selected techniques for prevention. Proc Am Assoc North Equine Pract, 52:270.

Steed, DL. (2004): Debridement. Am J Surg May, 187(5A):71S–4S.

Stotts, NA., and Wipke-Tevis, DD. (2001): Cofactors in impaired wound healing. In *Chronic wound care*; *A Clinical Source Book for the Healthcare Professional (3rd edition)*. Wayne PA, HMP Communications, p.265

Swaim, SF. And Bohling, MW. (2003): Miseau point surles récents développements en gestion de plaies chez les animaux de compagnie. Med Vet QC, 33:99.

Swaim, SF., Gillette, RL., Sartin, EA., (2000): Effects of a hydrolyzed collagen dressing on the healing of open wound, Am J Vet Res, 61:1574.

Swann, WJ. (2006): Improving the welfare of working equine animals in developing countries. Appl Anim Behav sci, 100: 148-151.

Taylor, DEM., Cooper, GJ., Evans, VA., (1986): Effect of hemorrhage on wound healing and its possible modification by 1-ethoxysilatrane; J Royal Coll Surg Edinburgh, 31:13

Theoret, C. (2006): Wound repair. In JA Auer, JA Stick eds. *Equine surgery (3rd edition),* Philadelphia; Saunders/Elsevier, p.44.

Theoret, CL. (2005): Wound repair and specific reaction to injury. In JA Auer and JA Stick eds. Equine surgery (3rd edition).Philadelphia: W.B. Saunders, p. 44.

Theoret, CL., Barber, SM., Moyana, TN. (2001): Expression of transforming growth factor 3, and basic fibroblast growth factor in full-thickness skin wounds of equine limbs and thorax; Vet Surg, 30:269

Thomas, S., Fisher, B., Fram, P. and Waring, M. (2012): Odour Absorbing Dressings: A comparative laboratory study. World Wide Wounds [serial on the Internet]; 1998.[Cited February]. Available from: http://www.worldwidewounds.com/1998/march/Odour-Absorbing-Dressings/odour-absorbing dressings.html.

Trengrove, NJ., Stacey, MC., MacAuley, S. (1999): Analysis of the acute and chronic wound environments; The Role of Proteases and their Inhibitors, Wound Rep Regen, 7:442

Velmahos, GC., Vassiliu, P., Demetriades, D. (2002): Wound management after colon injury; Open or closed? A prospective randomized trial, Am Surg, 68:795.

Walton, GS., Neal, PA.(1972): Observations on wound healing in the horse, Equine Vet J, 4:93.

Wilmink, JM., Nederbragt, H., van Weeren, PR. (2001): Differences in wound contraction between horses and ponies: the in vitro contraction capacity of fi broblasts. Equine Vet J, 33:499

Wilmink, JM., Stolk, PW., van Weeren, PR. (2000): The effectiveness of the haemodialysate Solcoseryl for second-intention wound healing in horses and ponies, J Vet Med A Physiol Pathol Clin Med, 47:311.

Wilmink, JM., van Herten, J., van Weeren, PR. (2002): Retrospective study of primary intention healing and sequestrum formation in horses compared to ponies under clinical circumstances, Equine Vet J, 34:270.

Wilmink, JM., van Weeren, PR., Stolk, PW. (1999): Differences in second-intention wound healing between horses and ponies; Histological aspects, Equine Vet J, 31:61.

Wilmink, JM., Veenman, JN., van den Boom, R. (2003): Differences in polymorphonucleocyte function and local inflammatory response between horses and ponies, Equine Vet J, 35:561.

Wysocki, AB., Staiano-Coico, L., Grinnell, F. (1991): Wound fluid from chronic leg ulcers contains elevated levels of metalloproteinases MMP-2 and MMP-9; J Invest Dermatol, 101:64

Zanette, C., Pelin, M., Crosera, M. (2011): Silver nanoparticles exert a long lasting anti-proliferative effect on human keratinocyte HaCaT cell line; Toxicol in Vitro. Aug, 25(5):1053–60.

Zhong, SP., Zhang, YZ. And Lim, CT. (2010): Tissue scaffolds for skin wound healing and dermal reconstruction; Wiley Interdiscip Rev Nanomed Nanobiotechnol Sep–Oct, 2(5):510–25.

Zhou, J., Loftus, AL., Mulley, G. and Jenkins, AT. (2010): A thin film detection/response system for pathogenic bacteria, J Am Chem Soc May 12, 132(18):6566–70.

Zhu, WH., Guo, X., Villaschi, S. (2000): Regulation of vascular growth and regression by matrix metalloproteinases in the rat aorta model of angiogenesis, Lab Invest, 80:545.

2019 SCIENCELINE

Online Journal of Animal and Feed Research

Volume 9, Issue 2: 86-91; March 25, 2019

ISSN 2228-7701

In Vitro DIGESTIBILITY AND *In Situ* DEGRADABILITY OF SUGARCANE BAGASSE TREATED WITH UREA AS ENERGY SOURCE IN TOTAL MIXED RATION FOR GOAT

Balgees, A. ATTA ELMNAN and Reem, A. ISMEAL

Department of Animal Nutrition, Faculty of Animal Production, University of Khartoum, P. O. Pox 321, Khartoum, Sudan

Supporting Information

ORIGINAL ARTICLE

PII: S222877011900011-9

Received: February 18, 2019

Revised: March 20, 2019

ABSTRACT: This study was carried out to investigate the effect of replacing sorghum grains (SG) by sugarcane bagasse treated with 5% urea (SCBU) as energy source in total mixed ration. *In vitro* dry matter digestibility (IVDMD) and *in situ* DM degradability were adopted to conduct the experiment. In order to meet the nutrient requirement of goat, the following four iso-caloric and iso-nitrogenous rations were formulated: A= 0% SCBU (control); B= 2.6% SCBU replaced 10% SG; C= 5.2% SCBU replaced 20% SG; and D= 7.8% SCBU replaced 30% SG. The IVDMD was significantly (P≤0.05) affected by dietary level of SCBU which was decreased gradually with increasing SCBU levels in examined rations. Ration A gained the highest value of IVDMD (88.50%) followed by ration B (84%), C (78%) and D (74.50%). An *in situ* DM degradability results showed that the readily soluble fraction (a), slowly fermented material (b) and the potential degradability (PD) were significantly (P≤0.05) higher for ration A than rations B, C and D. Although DM digestibility and degradability of rations B, C and D were decreased with progress level of SCBU in the rations, the values obtained from present study might be considered within the range of moderate to high level of digestibility and degradability especially ration B which replaced 10% of SG. More research is needed to study the effect of replacing SG by SCBU regarding the nutrients intake, animal performance in an *in vivo* experiment and to determine its economic feasibility.

Keywords: Sorghum Grains, Bagasse, Urea, Energy, Total Mixed Ration

INTRODUCTION

To address problems associated with poverty and food shortage, scientists investigated alternative food sources, evaluated present land use and utilization of food, thus feeding grains to ruminants is questioned because man and monogastric can utilize grains directly. On the other hand ruminants are characterized by their ability to convert low quality roughage to high nutritive products, which are useful to man e.g. (meat, milk, natural fibers, leather and manure) (Gertenbach and Dugmore, 2004).

Agricultural by-products such as bagasse have enough potential to be used as non-conventional roughage for animal feed in Sudan, particularly when forages are in short supply. Bagasse is the main byproduct of sugarcane industry; it contains 60 to 70% carbohydrate, mostly in the form of polysaccharides and is a potential source of dietary energy for animals. The major limitation of bagasse as feed is its low digestibility which is due to association of lignin with cellulose and hemicelluloses (Atta Elmnan et al., 2007, 2009, 2011, 2015).

To improve the nutritive value of these agriculture residues, it is important to breakdown the linkages among cellulose and lignin by mechanical, chemical or biological and combined biological plus chemical treatments. Many scientists suggested the use of ammonia and urea to increase the crude protein contents of the poor quality roughages (Shoukry et al., 1992, Fouad et al., 1998, Atta Elmnan et al., 2007, 2009, 2011). However, chemically and physically treated crop residues and other poor quality forages have been investigated extensively throughout the last years; there have been limited research studied the inclusion of these treated materials in total mixed ration (TMR) in Sudan.

The aim of the present study was to investigate the possibility of replacing sorghum grains by 10%, 20% and 30% of treated baggasse, which represent 2.6%, 5.2% and 7.8% of total ration component, respectively. The specific objectives were to determine the effect of inclusion different levels of treated bagasse instead of sorghum grains on *in vitro* dry matter digestibility and *in situ* dry matter degradability.

To cite this paper: Elmnan B,A.Atta and Ismeal R,A (2019). *In vitro* digestibility and *in situ* degradability of sugarcane bagasse treated with urea as energy source in total mixed ration for goat. *Online J. Anim. Feed Res.*, 9(2): 86-91. www.ojafr.ir

MATERIALS AND METHODS

Experimental site

The experiment was conducted at the laboratory of department of animal nutrition, faculty of animal production, university of Khartoum, Sudan.

Feeds preparation

Four iso-caloric and iso-nitrogenous rations were formulated Table 1 to meet the daily nutrient requirement of goat according to NRC (1985). Table 2 showed the ingredients of the different rations which can be namely as follow: A= 0% SCBU (control); B= 2.6% SCBU replaced 10% SG; C= 5.2% SCBU replaced 20% SG; D= 7.8% SCBU replaced 30% SG.

Table 1 - Calculated Chemical Analysis of Experimental Rations

Term \ Treatments	A	B	C	D
CP (%)	12.92	12.86	12.92	12.57
ME (MJ/Kg DM)	10.43	10.08	9.81	9.59

A: 0% of treated bagasse, B: contained 2.6% treated sugarcane bagasse which replaced 10% of sorghum grains, C: contained 5.2% trea sugarcane bagasse which replaced 20% of sorghum grains and D: contained 7.8% treated sugarcane bagasse which replaced 30% of sorgh grains.

Table 2 - Ingredients (%) of the experimental diets

Term \ Treatments	A	B	C	D
Sorghum	26.00	23.40	20.80	18.20
Treated bagasse	0.00	2.60	5.20	7.80
Molasses	33.00	35.00	32.00	30.00
Groundnut cake	6.00	8.00	8.00	7.00
Wheat bran	34.00	30.00	33,00	36,00
Salt	0.50	0.50	0.50	0.50
Lime stone	0.50	0.50	0.50	0.50
Total	100	100	100	100

A: 0% of treated bagasse, B: contained 2.6% treated sugar cane bagasse which replaced 10% of sorghum grains, C: contained 5.2% treated su cane bagasse which replaced 20% of sorghum grains and D: contained 7.8% treated sugar cane bagasse which replaced 30% of sorghum grains.

Chemical Analysis

Samples of feed examined and residues were analyzed for their proximate components, dry matter (DM), ash, ether extract (EE), crude protein (CP), crude fiber (CF) according to AOAC (1990). While nitrogen free extract (NFE) calculating using the following equation: NFE% = DM – (CP% + EE% + ash% + CF %).

In- vitro Dry Matter digestibility

The two steps procedure was used for *in vitro* determination of digestibility of rations using four replicates to all treatments. Rumen liquor was collected by stomach tube from four mature goats. In the first step, dried rations were incubated in test tubes with rumen fluid. The tubes also contain buffer solution, macro-minerals, trace-minerals, nitrogen sources, and reducing agents to maintain pH and provide nutrients required for growth of rumen bacteria. Because oxygen is toxic to rumen bacteria, solutions are gassed with carbon dioxide to maintain anaerobic conditions, and temperature is held at 39°C (body temperature) during the incubation. In the second step, after 48 hours of incubation, an enzyme solution is added to stimulate digestion in the small intestine (Tilley and Terrie, 1963).

In situ dry matter degradability

Degradability study of was carried out in the rumen of two fistulated adult Nubian goat according to the nylon bag technique described by Ørskov et al., (1980). The fistulated animal was fed at maintenance level on a balanced roughage concentrate diet with free access to water and mineral blocks. The feeds were ground through a 3-mm sieve and three replicate samples of each, weighing about 5g, were put into nylon bags and incubated in the rumens of two fistulated adult Nubian goats to determine the degradability of the dry matter. The incubation periods were 4, 8, 16, 24, 48, 72 and 96 hrs Washing losses were determined in triplicate by weighing about 5g of each feed sample into nylon bags, soaking them in warm water (39° C) for an hour and subsequently washing the bags in a washing

To cite this paper: Elmnan B,A.Atta and Ismeal R,A (2019). *In vitro* digestibility and *in situ* degradability of sugarcane bagasse treated with urea as energy source in total mixed ration for goat. *Online J. Anim. Feed Res*., 9(2): 86-91. www.ojafr.ir

machine as was done for those incubated in the rumen and finally drying the samples in an oven (60°C) for 48h. The course of degradation of the feeds was described by using the equation of McDonald (1981), p -a + b (1 - e-ct).

Statistical analysis

Data obtained from experiment were subjected to analysis of variance (ANOVA) according to Completely Randomize Design. Where the F test was significant, Means between treatments were compared using the least significant difference (LSD).

RESULTS AND DISCUSSION

In-vitro dry digestibility of tested rations

The results of *in vitro* dry digestibility (IVDMD) was significantly affected (P≤0.05) by dietary level of treated sugarcane bagasse (SCBU) which was decreased gradually with increasing treated bagasse levels in the rations Table 3. Treatment A (0% SCBU) secured the highest value of IVDMD, in contrast ration D (7.8% SCBU) recorded the lowest value. This result implies that microbes in treatment A had high nutrients uptake, readily available energy and protein contents which might had improved microbial growth and fermentation (Chatterjee et al., 2006). Although, the four diets were iso-caloric and iso-nitrogenous the IVDMD was decreased with the increasing percentage level of treated bagasse, this may be due to longer retention time of the high fiber rations in association with low soluble carbohydrates since the SG was replaced by 10%, 20%, and 30% of SCBU for rations B, C and D respectively. There are several previous studies proved that the fiber fraction of a food had the greatest influence on its digestibility. Adewusi and Matthew (1994) and Atta Elmnan et al. (2007, 2009, 2011) reported that the increase of CF content in diet resulted in decreases in DM digestibility. The major limitation of bagasse as feed is its low digestibility which is due to association of lignin with cellulose and hemicelluloses (Atta Elmnan et al., 2009; 2011). Ensminger et al. (1990) reported that bagasse has high fiber content which resulted in low dry matter digestibility (25%). The same result was obtained by Jayasuriya (2000), Chappidi et al. (2014) who reported that the increase in the roughage component of the total mixed ration decreased the IVDMD. Similarly, Hindrchsen et al. (2002) concluded that the large proportion of lignified cell wall of fibrous materials leading to attachment difficulty by rumen microorganisms resulted in low DM digestibility.

As can be observed from the current study the IVDMD is very high than the result reported by Ensminger et al. (1990), this could be explained by the fact that bagasse in the present study was inclusion in total mix ration after treated with urea resultant in high IVDMD. Maximum benefits from agricultural/agro-industrial by-products as feed will be achieved when the deficient nutrients like energy, protein and minerals were supplemented to these materials which resulted in promoting the efficiency of rumen microbes' growth and thus increased digestibility (Aletor, 2010). Also Ahmed and Fadal Allseed (2007) reported that the higher fermentable carbohydrates, available nitrogen, better nutrient availability for rumen microbes was achieved when crop residues supplemented with concentrates.

Table 3. *In vitro* dry matter digestibility (%) of tested rations

Treatments	IVDMD%
A	89[a]
B	84[b]
C	78[c]
D	75[d]
SEM±	1.4

A: 0% of treated bagasse, B: contained 2.6% treated sugar cane bagasse which replaced 10% of sorghum grains, C: contained 5.2% treated sugar cane bagasse which replaced 20% of sorghum grains and D: contained 7.8% treated sugar cane bagasse which replaced 30% of sorghum grains. SEM standard error of means: a-d means with different superscripts in the same column were significantly different (P≤0.05).

In-situ DM degradability of tested rations

Figure 1 showed the result of *in-situ* degradability of the tested rations during incubation time which extended from 0 to 96 hours. There was an increase in DM disappearance associated with increasing incubation time, while the increasing level of treated bagasse associated with reduction in DM disappearance. Obviously the low degraded materials recorded by rations B, C and D could be justify by the high fiber content in these rations. Generally high crude fiber content of feed stuff which bound to the cell walls and thus mostly unavailable to microbial enzymatic to attack the feed, resulted in low degradability (Nherera et al., 1998; Atta Elmnan and Hawa, 2011; Atta Elmnan et al., 2007; 2009; 2011).

To cite this paper: Elmnan B,A.Atta and Ismeal R,A (2019). *In vitro* digestibility and *in situ* degradability of sugarcane bagasse treated with urea as energy source in total mixed ration for goat. *Online J. Anim. Feed Res.*, 9(2): 86-91. www.ojafr.ir

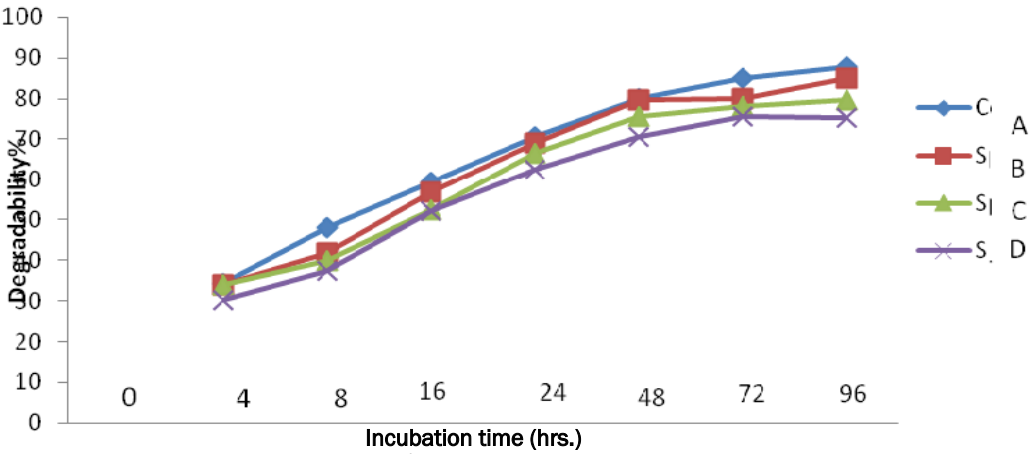


Figure 1. Rumen degradation of DM% of tested rations

Estimation of Kinetics degradability of tested rations

Table 4 showed the results of kinetic for *in-situ* degradability from different rations. The readily soluble fraction (a) was higher significantly (P≤0.05) for ration A which contain 0% of bagasse whereas rations B and C which contain 10 and 20 had moderate value of this fraction. Ration D that contained 30% recorded the lowest value of readily soluble fraction (a). A slowly fermented material (b) is measure the proportion of the feed that can be fermented in the rumen (Mupangwa et al., 1997), in the present study this fraction for different treatments can be ranked as follow rations A and B followed by rations C and D. The maximum value of potential degradability (PD) was recorded by ration A followed by rations B, C and D. The maximum value of PD for ration A may be due to its high content of readily degraded of soluble carbohydrates and N and low content of CF. Consequently the low value of PD recorded by B, C and D rations could be explained by their high CF and low ME content. There are negative correlation with rate of degradability as well as potential degradability and effective degradability of DM with the CF content (Smith et al., 1991, Ramana et al., 2000, Melkau et al., 2003, Kiran et al., 2007 and Atta Elmnan et al., 2013). Also Bakhashwain et al., (2010) demonstrated that the negative effect of cell wall content on PD could be due to the reduction of the microbial activity through increasing the adverse environmental conditions.

The four rations showed no differences in the rate of fermentation c among them which concluded the four rations can be considered as a good feedstuff for the animals feeding. The same fermentation rates indicate same nutrient availability for ruminal microorganisms (Mirzaeiet et al., 2011 and Fievezet et al., 2005). Although DM digestibility and degradability of rations B, C and D were decreased with progress level of treated bagasse in the rations, the values obtained from present study may considered within the range of moderate to high level of digestibility and degradability especially ration B (84. %) that replaced 10% of sorghum grains which can be used effectively during dry season.

Table 4 - Kinetics degradability of tested rations

Term	A	B	C	ED 0.02	ED 0.05	ED 0.08	PD
A	23.00^{a}	63.64^{a}	0.056^{a}	69.80^{a}	56.50^{a}	49.10^{a}	86.70^{a}
B	20.16^{b}	63.54^{a}	0.056^{a}	67.10^{b}	53.90^{b}	46.50^{b}	83.70^{b}
C	20.38^{b}	59.59^{b}	0.054^{a}	63.90^{c}	51.50^{c}	44.60^{c}	80.00^{c}
D	17.83^{c}	58.12^{b}	0.056^{a}	60.80^{d}	48.80^{d}	42.10^{d}	75.70^{d}
SEM	0.36	0.40	0.06	0.05	0.05	0.04	0.04

a: Readily degradable fraction; b: slow degradable fraction; a+b: Potential degradability; c: Rate of degradability; ED: Effective degradation at three levels of rumen out flow rate; A: 0% of treated bagasse, B: contained 2.6% treated sugarcane bagasse which replaced 10% of sorghum grains, C: contained 5.2% treated sugarcane bagasse which replaced 20% of sorghum grains and D: contained 7.8% treated sugarcane bagasse which replaced 30% of sorghum grains, SEM standard error of the mean: a-c means with different superscripts in the same column were significantly different (P≤0.05).

CONCLUSION

The values of DM digestibility and degradability of tested rations are within the range of moderate to high level of digestibility. Readily energy source must be added to treated bagasse in total mix ration to enhance ME content of treated bagasse which may improve digestibility. More study is needed to evaluate the treated sugarcane bagasse in total mix ratio in *in vivo* feeding trial and to approve the economic appraisal.

To cite this paper: Elmnan B,A.Atta and Ismeal R,A (2019). *In vitro* digestibility and *in situ* degradability of sugarcane bagasse treated with urea as energy source in total mixed ration for goat. *Online J. Anim. Feed Res*., 9(2): 86-91. www.ojafr.ir

DECLARATIONS

Corresponding Author

Balgees, A. Atta Elmnan; E-mail: balgeesatta@gmail.com

Author's contribution

Balgees AAE and Reem Al participated in the design of study. Reem Al performed the experiments. Balgees AAE analyzed the data, and critically revised the manuscript for important intellectual contents. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

REFERENCES

Adewusi SRA and Matthew OI (1994).Nutritional evaluation of spent grains from sorghum malts and maize grit. Plant Foods for Human Nutrients. (Formerly Qualitas Plantarum), 46: 41-51.

Ahmed GM and Fadal Allseed AMA (2007). Chemical composition and *in vitro* gas production characteristics of six fodder trees, leaves and seeds. Research Journal of Agriculture and Biological Sciences, 3: 983-986.

Aletor O (2010). Nutritive and physic-chemical characteristics of same plants and animal based protein concentrate. Journal of Chemical Sciences, 2(2): 155:165.

AOAC (1990). Association of Official Analytical Chemists. Official Methods of Analysis, 15th Edition. Washington D.C. pp. 69-88.

Aregheore EM and Perera D (2004). Effects of Erthrinavariegata, Gliricidiasepium and Leucaenaleucocephala on dry matter intake and nutrient digestibility of maize stover before and after sparing with molasses. Animl Feed Science and Technology, 11: 190 - 201.

Atta Elmnan AB, Fadel Elseed AMA and Salih AM (2007). Effect of Ammonia and Urea Treatments on the Chemical Composition and Rumen Degradability of Bagasse. Journal of Applied Sciences Research, 3: 1359-1362.

Atta Elmnan AB, Fadal Allseed AMA and Salih AM (2009). Effects of *Albizia Lebbeck* or Wheat Bran Supplementation on Intake, Digestibility and Rumen Fermentation of Ammoniated Bagasse. Journal of Applied Scienes Research, 5(8): 1002-1006.

Atta Elmnan BA, Fadal Elseed AMA and Salih AM (2011). Effect of supplementing a basal diet with treated or untreated bagasse with different level Albizia Lebback on intake, digestibility, and rumen fermentation. Pakistan Journal of Nutrition, 10 (12): 1149-1153.

Atta Elmnan BA and Hawa MD (2011). Nutritive Evaluation of Some Pasture Plants in Early and Late Rainy Season in Mosai (Southern Darfur State). Australian Journal of Basic and Applied Sciences, 5(12): 2065-2070.

Atta Elmnan AB, FadalElseed AMA, Mahala AG and Amasaib EO (2013). In-situ degradability and *In vitro* Gas production of selected multipurpose tree leaves and Alfalfa as ruminant feeds. Worlds Veterinary Journal, 3(2):46-50.

Bakhashwain A, Sallam SMA, Allam AM (2010). Nutritive value assessment of some Saudi Arabian foliage by gas production technique in vitro. JKAU: Met., Env. & Arid Land Agric. Sci, 21 (1) : 65-80.

Chappidi V, Seshaiah Y, Ramana Reddy S, Jagadeeswara R and Srivani M (2014). Prediction of optimum roughage to concentrate ration in sweet sorghum (sorghum bicolor L.Moench) bagasse based total mixed ration for buffaloes using *in vitro* gas technique. Journal of Advanced Veterinary Animal Research, 1(4):244-227.

Chatterjee PN, Kamra DN and Neeta A (2006). Effect of roughage source, protein and energy levels on *in vitro* fermentation and methanogenesis. Journal of Science Advanced and Animal Search, 2(4): 245-225.

Ensminger ME, Oldfield JE and Heinemann WW (1990). Feeds and nutrition.2th ed. Ensminger Publishing company. California, USA, p.319.

Fievez V, Babayemi OJ and Demeyer D (2005). Estimation of direct and indirect gas production that requires minimal laboratory facilities. Animal Feed Science and Technology, 123-124:197-210.

Fouad RT, Deraz TA and Atiat Ismail SA (1998). Biological versus urea treatment of roughages for sheep. Journal of Agricultural Science Mansoura University, 23:103.

Gertenbach WD and Dugmore TJ (2004). Crop residues for animal feeding. SA-ANIM SCI, 5: 49-51.

Hindrichsen IK, Osuji PO, Odenyo AA, Madsen J and Hvelplund T (2002). Effects of supplementation of basal diet of maize stover with different amounts of Luciana diferesifolia on intake, digestibility nitrogen metabolism and rumen parameters in sheep. Animal Feed Science and Technology, 98(3): 131-141.

Jayasuriya MCN (2000). Principles of ration formulation for ruminants. Proceedings of the final review and planning meeting of an IAEA Technical Cooperation Regional AFR Project organized by joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, Cairo, Egypt; pp 25-29.

Kiran D and Krisshnamoorthy U (2007). Rumen fermentation kinetic and nitrogen degradability of commonly used ruminant feedstuffs in Vitro. Animal Nutrition and Feed Technology, 7(1): 963-2972.

Melkau S, Petes J and Tegegne A (2003). *In vitro* and *in situ* evaluation of selected multipurpose trees, wheat bran and Lablab purpurious as potential feed supplemented to leaf straw. Animal Feed Science and Technology, 108:159-179.

Mirzaei AA, Maheri N, Mansouri H, Razeghi MEAR, Aghajanzaeh GA and Alipoor K (2011). Estimation of the nutritive value of tomato pomace for ruminant using *in vitro* gas production technique. African Journal Biotechnology, 10(33):6251-6256.

Mupangwa JF, Ngongoni NT and Topps JH (1997). Chemical composition and dry matter of forage legumes cassia rotundiforlia cv,wynn,lablab purpureus cv,highworth and macroptilium atropurpureum. PNdlovu. Animal.Feed science Technolgy, 17: 22:123.

Nherera FV, Ndlovu LR and Dzowela BH (1998). Utilization of leucaena diversfolia, Leucaena esculenta, Leucaena pallid and Calliandra calothyrsus as nitrogen supplements for growing goats fed maize stover. Animal Feed Science and Technology, 74(1): 15-28

NRC (1985). Nutrient requirements of domestic animal. No. 5. Nutrient requirements of goat, 5th ed. National Academy of Sciences, Washington, D.C. USA.

Ørskov ER, Hovell FD and Mould F (1980). The use of nylon bag technique for the evaluation of feed stuff. Tropical Animal Production, 5: 195- 213.

Ramana DBV, Sultan S, Solanki KR and Nagi AS (2000). Nutritive evaluation of some nitrogen and non nitrogen fixing multiple purpose tree species. Animal Feed Science and Technology,88:103-111.

Shoukry MM (1992). Effect of urea treatment on chemical composition, *in vitro* dry matter disappearance and digestibility of dry matter and cellwall constituents of some poor quality roughages. Annals of agricultural science Moshtohor Journal, 30 (2):677.

Smith OB, Idou OA, Asaolu VO and Odunlami O (1991). Comparative rumen degradability for forage browse, crop residues, agricultural by products. Livestock Research for Rural Development, 3:2.

Tilley JMA and Terry RA (1963). A Two-stage Technique for *in Vitro* digestion of Forage Crops. Journal of the British Grassland Society, 18:104.

To cite this paper: Elmnan B,A.Atta and Ismeal R,A (2019). *In vitro* digestibility and *in situ* degradability of sugarcane bagasse treated with urea as energy source in total mixed ration for goat. *Online J. Anim. Feed Res*., 9(2): 86-91. www.ojafr.ir

2019 SCIENCELINE

Online Journal of Animal and Feed Research

Volume 9, Issue 2: 92-102; March 25, 2019

ISSN 2228-7701

CHEMICAL COMPOSITION AND SENSORY EVALUATION OF MEAT OF *Tswana* HENS FED DIFFERENT PROTEIN SOURCES: MOPANE WORM, BAMBARA GROUNDNUT AND MORAMA BEAN

Freddy MANYEULA ✉; Christopher Mareledi TSOPITO[1], Kebadire MOGOTSI[2], Shalaulani James NSOSO[1], John Mashrimo KAMAU[1] and John Cassius MOREKI[3]

[1]*Department of Animal Science and Production, Botswana University of Agricultural and Natural Resources, Private Bag 0027, Gaborone, Botswana*
[2]*Department of Crop Science, Botswana University of Agricultural and Natural Resources, Private Bag 0027, Gaborone, Botswana*
[3]*Department of Corporate Services, Ministry of Agricultural Development and Food Security, Private Bag 003, Gaborone, Botswana*

✉*Supporting Information*

ORIGINAL ARTICLE
PII: S222877011900012-9
Received: January 29, 2019
Revised: March 20, 2019

ABSTRACT: **This study investigated the chemical composition and sensory evaluation of meat of *Tswana* hens fed diets containing mopane worm (*Imbrasia belina,* Westwood) or Bambara groundnut (*Vigna subterranea* (L) Verde) or morama bean (*Tylosema esculentum,* Burchell A. Schreiber) as different protein sources under intensive management system. Sixty Tswana hens (25 weeks old) were bought from a local farmer and reared up to 38 weeks of age on diets containing *T. esculentum* or *V. subterranea* or *I. belina* and a commercial layer diet as a control. Experimental diets met the nutritional composition of control diet. Whole thighs, drumsticks obtained from the carcasses at week 28, 33 and 38 were deboned. The thigh, drumstick meat and bone were analysed for phosphorus (P), calcium (Ca) and potassium (K) using AOAC methods. Furthermore, boiled 2 x 5 cm portions of drumsticks, breasts and thighs were organoleptically assessed for flavour, odour, juiciness, tenderness and firmness by untrained panelists (15 males and 15 females) using a 5-point Likert scale. Bone P and Ca were high in all treatment diets at 28 weeks compared to 38 weeks of age. The meat DM, CP, P, Ca and K and bone P, Ca and K contents were not significantly affected by treatment diets. Organoleptic quality from all treatment groups and across different ages received moderate (3.52) to good rating (4.13). In conclusion, *Imbrasia belina* (Mopane worm) or *Vigna subterranea* (Bambara groundnut) or *Tylosema esculentum* (Morama bean) can replace soybean meal in *Tswana* hens without compromising meat and bone chemical composition and also organoleptic qualities.**

Keywords: **Crude protein, Minerals, Organoleptic Attributes, Protein Sources, *Tswana* hens**

INTRODUCTION

The main constituents of layer meat are water, protein and fat (FAO, 2003). Increases in collagen and elastin, the proteins forming the connective muscle tissues are related to the toughness of the meat of layer birds. Age and sex of a bird influence the fat content, as does a high caloric diet. Layer meat provides a good source of high quality protein, iron and phosphates and the B vitamins (riboflavin and niacin). The vitamin content of the meat is influenced to a large degree by the vitamin content of the feed consumed (FAO, 2003).

Meat quality is a generic term used to describe properties and perception of meat that includes attributes such as carcass composition and the eating quality (Maltin et al., 2003). Consumer's sensory evaluation of eating quality takes into account tenderness, juiciness and flavour of meat as the most important elements. Tenderness and juiciness contribute to meat texture and form the basis for poultry meat quality (Dransfield, 1995). The quality of meat is changed from birth and through the growth period until the meat is mature enough for consumption.

Diet is an important factor in meat quality since it determines the quantity of fat that in turn affects meat flavour (Sokołowicz et al., 2016). Feed costs per unit increase in body weight are of great practical and economic importance in assessing the productivity of poultry meat. The major challenge of producing poultry meat goes to the cost of dietary protein (Rezaeipour et al., 2016); hence reduction of feed costs is a major concern for poultry producers. Poste (1990) stated that replacement of component of a maize soybean diet with a locally available alternative requires the assessment not only of the poultry performance and carcass chemical characteristics but also of the resulting meat quality. Since *Imbrasia belina* (Mopane worm) or *Vigna subterranea* (Bambara groundnut) or *Tylosema esculentum* (Morama bean) are consumed by human being, it could be assumed that these protein sources should also be an ingredients that could be included successfully in Tswana hen's diets.

To cite this paper: Manyeula F, Tsopito Ch-M, Mogotsi K, Nsoso Sh-J, Kamau JM and Moreki JC (2019). Chemical composition and sensory evaluation of meat of *Tswana* hens fed different protein sources: Mopane worm, Bambara groundnut and Morama bean. *Online J. Anim. Feed Res.*, 9(2): 92-102. www.ojafr.ir

The protein content of Morama bean is 31.4 % while the oil content is twice as high as that of soya bean and is comparable to that of groundnut (Bower et al. 1988). Bambara groundnut is an indigenous African crop grown primarily for its seeds which are eaten fresh when semi ripe, as a pulse when dry and mature, or ground into a flour. Its crude protein is 17-25% (Belewu et al. 2008). Madibela et al. (2009) reported protein content of mopane worm to be about 56.8%. There is little information on chemical composition and organoleptic attributes of meat from *Tswana* hens fed diets formulated using local ingredients as protein sources under intensive system in Botswana. Therefore, a study was undertaken to investigate the composition and sensory evaluation of meat of hens fed diets with *Imbrasia belina* (mopane worm) or *Vigna subterranea* (Bambara groundnut) or *Tylosema esculentum* (Morama bean) as different protein sources.

MATERIALS AND METHODS

Study area

The experiment was conducted at the Botswana College of Agriculture (now Botswana University of Agriculture and Natural Resources), Content farm at Sebele, located 10 km north of Gaborone (the capital city of Botswana) from February to May 2010. The study site is located on 25°56' 29.1" east and 24°35'18.8" south at an altitude of 983 metres above sea level (Manyeula et al., 2018). The average annual rainfall is 450 mm and average daily temperature 30°C (Aganga and Omphile, 2000).

Birds and management

A total of 60 normal feathered *Tswana* hens (15 birds per treatment) aged 25 weeks (point of lay) were fed diets containing *I. belina*, *T. esculentum*, *V. subterranea* and a commercial layer diet (control) up to 38 weeks of age. The hens were reared intensively and supplemented with maize before the start of the study. At the beginning of the study, hens were individually weighed and tagged, then were vaccinated against Newcastle disease and infectious bursal disease (IBD). Clean water and feed were provided *ad libitum* for 90 days. Birds were raised on a deep litter system and received 16 hours of light daily throughout the experimental period.

Birds and slaughter

Three *Tswana* hens from each treatment (one from each replicate) were slaughtered at 28, 33 and 38 weeks of age. A humane slaughter method was carried out at Botswana University of Agriculture and Natural Resources (BUAN) slaughter facility, which involved rendering the birds insensible by electrical stunning and thereafter cutting jugular vein to allow for bleeding. Following dressing, the carcasses were individually packed in polythene bags and kept in the freezer at -17°C for proximate and mineral analyses and sensory evaluation.

Preparation of experimental diets

Chemical analysis of the diets: Prior to diet formulation, proximate analysis of *I. belina*, *V. subterranea* and *T. esculentum* were determined by the method of AOAC (2005). Calcium (Ca) was analysed using Inductively Coupled Plasma Mass optical Emission Spectrometer (Optimal 2100DV Model) while phosphorus (P) was determined using Kjeldahl digestion by ultraviolet spectrophotometer (Shimadzu UV 160 pc model) (AOAC, 2005). Table 1 presents the chemical composition of the protein sources used in the formulation of diets (%DM).

The protein sources were ground and mixed with other ingredients to formulate their respective mash diets. Diets were isonitrogenous (16% CP) and isocaloric (13 MJ/Kg) and were formulated to meet the nutritional value of the control diet (commercial layer diet) as recommended by National Research Council (1996). The *T. esculentum* and *V. subterranea* diets were supplemented with DL methionine and lysine to ensure that these amino acids were not limiting for egg production and quality. The feed mixer auger (pet 14, animal shredder hammer mill foliage TRF 600) was used to mix different diets homogeneously. Ingredients used in the formulation of the different diets are shown in Table 2.

Diet formulation

Three experimental diets containing either roasted *I. belina* or dehulled *T. esculentum* and also dehulled *V. subterranea* as sources of protein were formulated using the Feed Mixer Computer Software (OSUNRC2002 model). Roasted *I. belina* was purchased from local farmers in Tsetsebye in Central district, dehulled *T. esculentum* from Letlhakeng in Kweneng district and dehulled *V. subterranea* was also purchased from the Botswana Agricultural Marketing Board in Gaborone.

Bone and meat chemical analysis: The right hand thigh and drumstick portions were cut from the frozen chickens and deboned for meat and bone chemical analyses. Analyses were conducted for laboratory dry matter (DM; AOAC method no 930.15), organic matter (OM; AOAC method no 924.05) and crude protein (CP; AOAC method no

To cite this paper: Manyeula F, Tsopito Ch-M, Mogotsi K, Nsoso Sh-J, Kamau JM and Moreki JC (2019). Chemical composition and sensory evaluation of meat of *Tswana* hens fed different protein sources: Mopane worm, Bambara groundnut and Morama bean. *Online J. Anim. Feed Res.*, 9(2): 92-102. www.ojafr.ir

984.13). Bone and meat P was determined using UV spectrophotometer (Shimadzu UV 160 pc Model) while, bone and meat Ca and K were analysed using Inductively Coupled Plasma Mass optical Emission Spectrometer (Optimal 2100DV model).

Taste panel evaluation

A day after slaughter of chickens, drumsticks, breasts and thighs were obtained, cut and cooked for one hour by boiling in water (Lewko et al., 2017). Thereafter, meat portions were cut into pieces of about 2 x 5 cm cubes and given to untrained panelists (15 males and 15 females) from BUAN to evaluate. The panellists were given guidelines on meat attributes and were asked to assess cooked meat sample for the six organoleptic attributes (i.e., odour, odour intensity, flavour, juiciness, tenderness and firmness) on Likert scale of 1 to 5, where odour was ranked from offensive to natural; odour intensity from odourless to strong; flavour from tasteless to very good; juiciness from very dry to very juicy, tenderness from very tough to very tender and firmness from very soft to very firm (Levie, 1979).

Table 1 - Chemical composition of *Zea mays (Z. mays*), *T. esculentum, V. subterranea* and *I. belina* used in the present study.

Parameter	*Z. mays*	*T. esculentum*	*V. subterranea*	*I. belina*
Dry matter (%)	79.32±1.4	90.12±4.2	89.32±5.7	91.36±8.5
Crude protein (%)	7±0.14	30±1.6	18±0.7	54±3.7
Crude fibre (%)	1.0±0.14	17±3.2	4.1±0.02	8.1±0.01
Crude fats (%)	4.1±0.07	40±2.3	5.9±0.5	13.9±2.1
Ash (%)	0.7±0.07	1.2±0.2	1.1±0.2	5.4±0.1
Calcium (%)	0.6±0.01	0.61±0.03	0.40±0.07	0.7±0.01
Phosphorus (%)	0.3±0.02	0.46±0.01	0.65±0.002	0.45±0.02
Gross energy (MJ/kg)	1.638±0.02	1.993±0.04	2.440±0.01	1.713±0.009

Table 2- Ingredients and nutrients composition of experimental diets formulated from *T. esculentum, V. subterranea* and *I. belina* used in the present study

Ingredients	*T. esculentum*	*V. subterranea*	*I. belina*	Control
Yellow maize (%)	59	19	78	*
T. esculentum (%)	37	0	0	*
V. subterranea (%)	0	77	0	*
I. belina (%)	0	0	18	*
Dicalcium phosphate (%)	1.2	1.1	1.2	*
Limestone (%)	1.1	1.2	1.1	*
Iodized salt (%)	0.4	0.4	0.4	*
Vat. Mineral premix (%)	0.1	0.1	0.1	*
DL-methionine (98%)	0.6	0.6	0.6	*
Lysine HCL (%)	0.6	0.6	0.6	0.6
Calculated analysis				
Met. energy (MJ/kg) [1]	13.68	13.38	13.18	13
Crude protein (%)	16.01	16.09	16.1	16
Calcium (%)	3.54	3.51	4.21	4.5
Phosphorus (%)	0.91	0.82	0.99	0.5
Determined analysis				
Dry Matter (%)	93.80±4.2	93.84±3.9	93.84 ±5.1	84±2.5
Crude protein (%)	15.96±1.02	16.00±2.0	16.80±1.4	16±0.8

*= Company confidential information. [1]Estimated using a method by Oduguwa et al. (2000) that is: ME (Kcal/kg) = 37 x % CP + 81.8 x % EE + % NFE. 1 caloric = 4.184 joules

Statistical analysis

Chemical composition and sensory evaluation data were analysed using PROC GLM procedure of SAS (2010) as a completely randomized design. Bone and meat chemical composition data were analysed according to the following model: $Y_{ijk} = \mu + D_i + W_j + (D \times W_{ij}) + E_{ij} + S_{ijk}$

Where $Y_{ijk} =$ response variables, μ = population mean, D_i = treatments effects, W_j = week of age, $(D \times W)_{ij} =$ interaction of age and diets, E_{ij} = experimental error, S_{ijk} = Sampling error. Differences among treatment means were determined using the PDIFF option in the LSMEANS statement of the GLM procedure of SAS (2010). Sensory evaluation data were analysed using non-parametric multivariate analysis. The level of significance for both chemical, proximate composition and sensory evaluation was set at P<0.05.

To cite this paper: Manyeula F, Tsopito Ch-M, Mogotsi K, Nsoso Sh-J, Kamau JM and Moreki JC (2019). Chemical composition and sensory evaluation of meat of *Tswana* hens fed different protein sources: Mopane worm, Bambara groundnut and Morama bean. *Online J. Anim. Feed Res.*, 9(2): 92-102. www.ojafr.ir

RESULTS

Repeated measures analysis showed significant (P<0.05) week × diet interaction effect on bone P and Ca with the exception of bone K (Table 3). Hens fed *V. subterranea* diet had the lowest (P<0.05) bone P concentration in all ages. At week 28, there was no dietary effect (P>0.05) on the concentration of P in the bone. Hens fed *V. subterranea* diet had lowest bone P contents in week 33. However, on week 38, hens fed *T. esculentum* and *I. belina* diets had higher (P<0.05) bone P content compared to other diets. Age (weeks) did not significantly affect bone Ca contents. Treatment diets did not significantly affect bone Ca contents at week 28 and 38. However, highest bone Ca contents was observed on hen fed *T. esculentum* diets at week 33. For bone K contents, there were neither age (weeks) nor treatment diet effects.

Dietary treatments and age (weeks) did not influence (P>0.05) DM, CP, P, Ca and K contents of the meat. Meat from hens fed *V. subterranea* diet had significantly lower odour than those fed control diet but there was no significant difference in odour in meat from hens fed control diet, *T. esculentum* and *I. belina* diet at 28 weeks of age (Figure 1). At 33 and 38 weeks, diets did not influence (P>0.05) odour. Similarly, age (weeks) also did not affect odour in all diets. Generally, consumer panellists ranked the meat odour between 3.52 and 4.13 (i.e., moderate to good) in all treatment diets and across the ages. Dietary treatments and age (week) did not influence (P>0.05) meat odour intensity, juiciness and tenderness for the duration of the study (Figures 2 to 4). Generally, consumer panellists ranked the meat odour intensity, juiciness and tenderness moderate (3.52) in all treatment diets and across the ages. There was no difference (P>0.05) in flavour between hens fed treatment diets at 28 and 38 weeks of age. However, at week 33 meat from hens fed *I. belina* diet had significantly moderate (low) flavour compared to hens on control and *V. subterranea* diets.

Also, no differences (P>0.05) in flavour between hens fed *T. esculentum* and *I. belina* diets were observed (Figure 5). Similarly, there were no significant (P>0.05) differences in meat flavour in all weeks from hens fed control, *V. subterranea* and *T. esculentum* diets. However, meat from hens fed *I. belina* diet had significantly (P<0.05) higher flavour at week 28 compared to 33 and 38 weeks.

Diets did not influence (P<0.05) firmness at week 28 and 38. However, at week 33 meat from hens fed *V. subterranea* diets was more (P<0.05) firm compared to that of hens on control diet. Hens fed control, *T. esculentum* and *I. belina* diets had similar (P<0.05) firmness at week 33 (Figure 6). Age (weeks) also did not affect firmness in the entire study periods. Generally, consumer panellists ranked the meat firm to slightly firm in all treatment diets and across the ages.

Table 3 - Mineral composition (%) of bone of *Tswana* hens fed three protein sources (*I. belina, T. esculentum and V. subterranea*) raised under intensive system from 28 to 38 weeks of age

Parameters	Age (weeks)	Control	*V. subterranea*	*T. esculentum*	*I. belina*
Phosphorus	28	19.5 ± 0.6^{ax}	14.3 ± 0.6^{bx}	19.7 ± 0.6^{ax}	18.0 ± 0.6^{ax}
	33	17.3 ± 0.6^{ay}	15.0 ± 0.6^{bx}	16.3 ± 0.6^{by}	16.3 ± 0.6^{by}
	38	15.7 ± 0.6^{ax}	14.7 ± 0.6^{bx}	16.3 ± 0.6^{ay}	16.3 ± 0.6^{ay}
Calcium	28	45.1 ± 2.0^{ax}	42.7 ± 2.0^{ax}	45.4 ± 2.0^{ax}	41.7 ± 2.0^{ax}
	33	37.4 ± 2.0^{bx}	36.8 ± 2.0^{bx}	41.7 ± 2.0^{ax}	39.8 ± 2.0^{bx}
	38	37.0 ± 2.0^{ax}	38.0 ± 2.0^{ax}	48.7 ± 2.0^{ax}	37.0 ± 2.0^{ax}
Potassium	28	2.70±0.6	1.50±0.6	2.20±0.6	2.50±0.6
	33	1.50±0.6	1.90±0.6	2.60±0.6	2.40±0.6
	38	3.00±0.6	2.10±0.6	2.90±0.6	2.10±0.6

^{ab}Values on the same row within the same parameter and different superscripts are significantly (P<0.05) different. ^{xy}Values on the same column within the same parameter and with different superscripts are significantly (P<0.05) different

Table 4 - Mineral and proximate composition (%) of meat of *Tswana* hens fed three protein sources (*I. belina, T. esculentum and V. subterranea*) raised under intensive system from 28 to 38 weeks of age

Parameters	Age (weeks)	Control	*V. subterranea*	*T. esculentum*	*I. belina*
Dry matter	28	29.5±6.3	30.6±6.3	30.4±5.1	31.5±6.3
	33	28.4±5.1	28.8±5.1	26.2±5.1	30.6±5.1
	38	28.1±5.1	30.5±5.1	30.9±5.1	30.6±5.1
Crude protein	28	82.7±7.7	80.4±7.7	83.1±7.7	82.0±7.7
	33	78.0±7.7	73.0±7.7	56.7±7.7	78.0±7.7
	38	81.2±7.7	75.8±7.7	76.0±7.7	76.2±7.7
Phosphorus	28	3.1±0.6	3.1±0.6	2.7±0.6	3.2±0.6
	33	3.0±0.6	3.0±0.6	2.7±0.6	2.9±0.6
	38	3.7±0.6	4.2±0.6	3.5±0.6	3.1±0.6
Calcium	28	4.2±0.7	2.8±0.3	2.5±0.1	2.9±0.1
	33	3.2±1.3	3.0±2.2	3.0±1.7	3.1±0.6
	38	3.9±1.5	4.2±1.7	3.6±1.0	2.7±0.4
Potassium	28	3.1±0.6	3.1±0.6	2.7±0.6	3.2±0.6
	33	3.0±0.6	3.0±0.6	2.7±0.6	2.9±0.6
	38	3.7±0.6	3.1±0.6	3.5±0.6	3.1±0.6

Values in the same column within the same parameter and with different superscripts are significantly (P<0.05) different. Values in the same row within the same parameter and with different superscripts are significantly (P<0.05) different

To cite this paper: Manyeula F, Tsopito Ch-M, Mogotsi K, Nsoso Sh-J, Kamau JM and Moreki JC (2019). Chemical composition and sensory evaluation of meat of *Tswana* hens fed different protein sources: Mopane worm, Bambara groundnut and Morama bean. *Online J. Anim. Feed Res.*, 9(2): 92-102. www.ojafr.ir

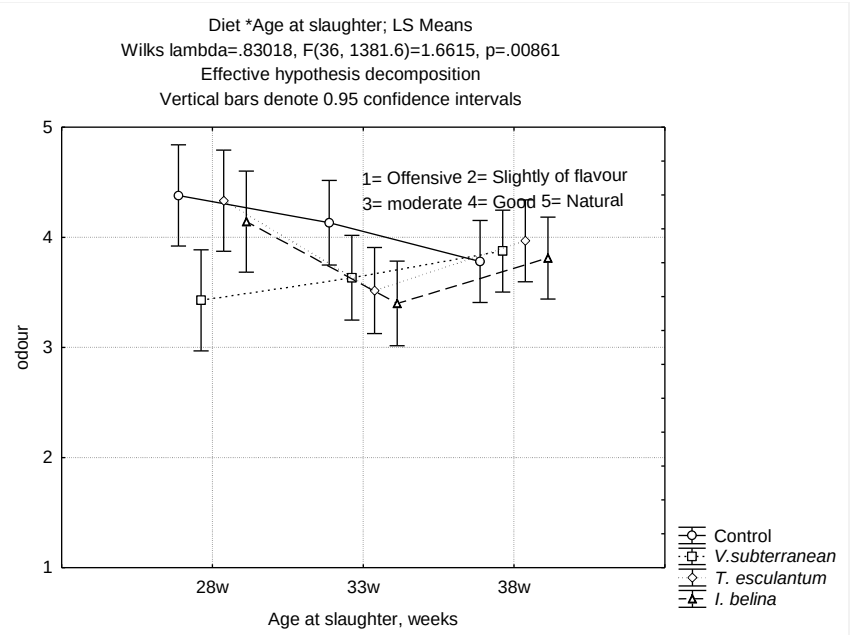


Figure 1 - Odour of meat of *Tswana* hens fed treatment diets at different ages at slaughter

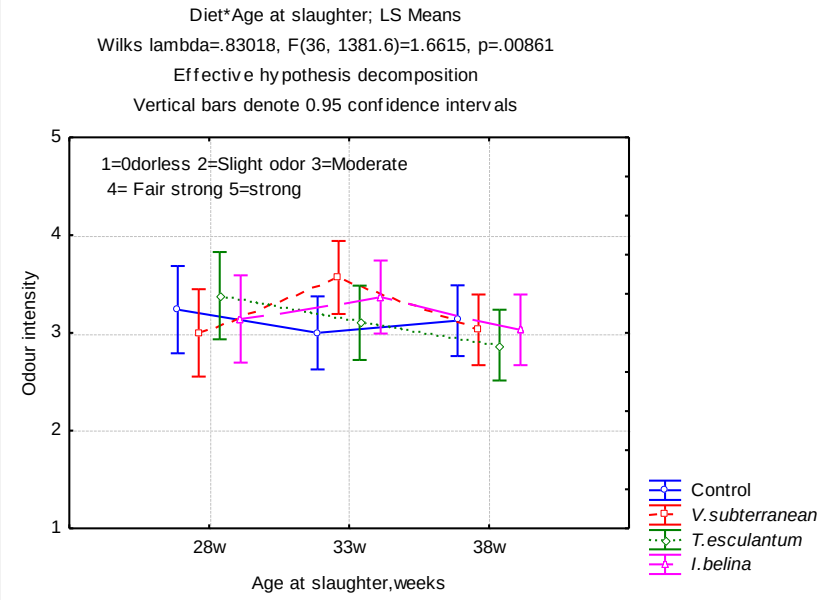


Figure 2 - Odour intensity of meat of *Tswana* hens fed treatment diets at different ages at slaughter

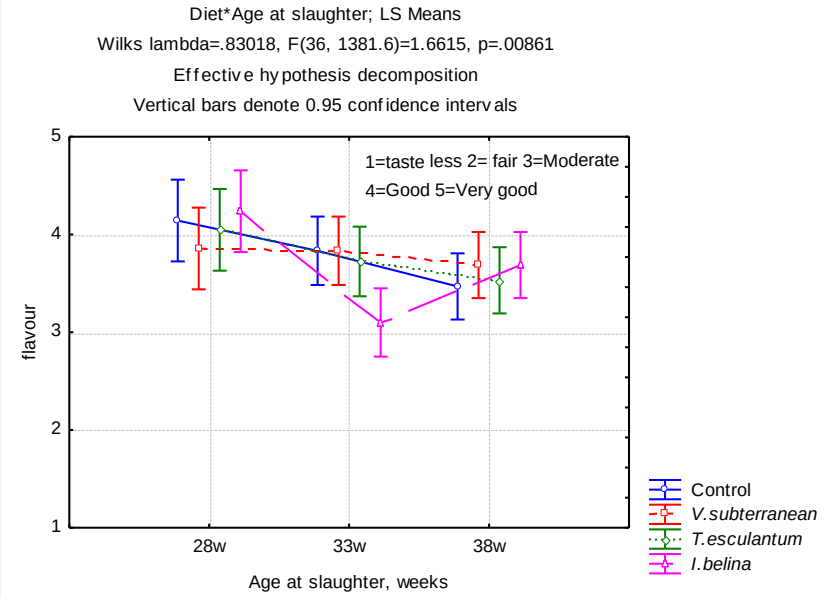


Figure 3 - Flavour of meat of *Tswana* hens fed treatment diets at different ages at slaughter

To cite this paper: Manyeula F, Tsopito Ch-M, Mogotsi K, Nsoso Sh-J, Kamau JM and Moreki JC (2019). Chemical composition and sensory evaluation of meat of *Tswana* hens fed different protein sources: Mopane worm, Bambara groundnut and Morama bean. *Online J. Anim. Feed Res.*, 9(2): 92-102. www.ojafr.ir

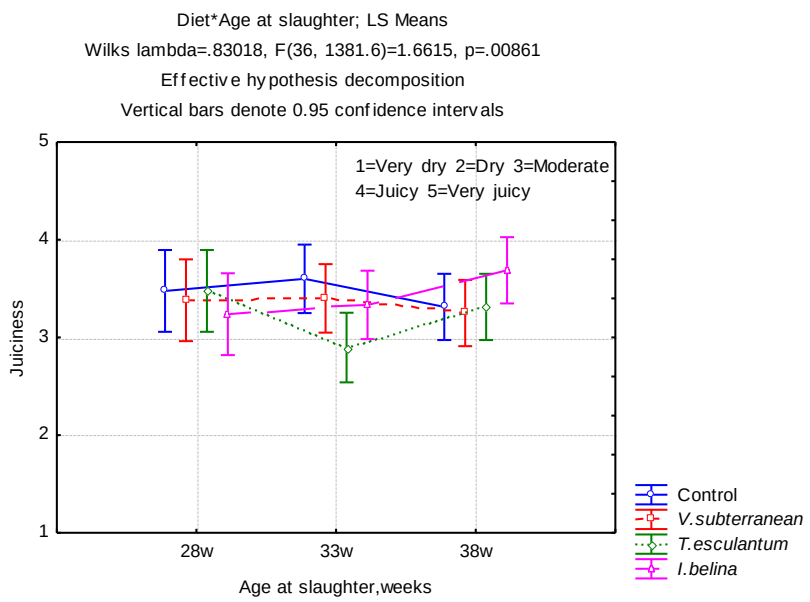


Figure 4 - Juiciness of meat of *Tswana* hens fed treatment diets at different ages at slaughter

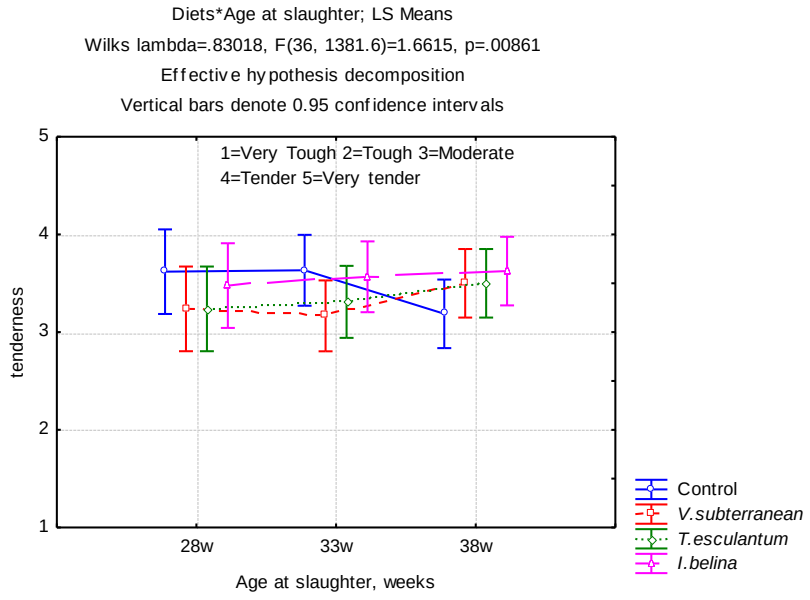


Figure 5 - Tenderness of meat of *Tswana* hens fed treatment diets at different ages at slaughter

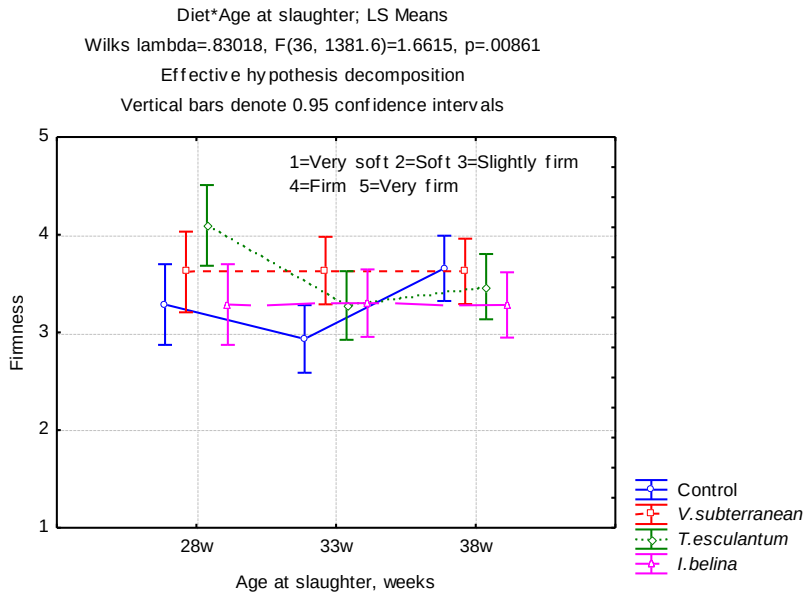


Figure 6 - Firmness of meat of *Tswana* hens fed treatment diets at different ages at slaughter

To cite this paper: Manyeula F, Tsopito Ch-M, Mogotsi K, Nsoso Sh-J, Kamau JM and Moreki JC (2019). Chemical composition and sensory evaluation of meat of *Tswana* hens fed different protein sources: Mopane worm, Bambara groundnut and Morama bean. *Online J. Anim. Feed Res.*, 9(2): 92-102. www.ojafr.ir

DISCUSSION

Bone P content of *Tswana* hens fed control, *T. esculentum* t and *I. belina* diets in the current study is consistent with the findings of Moreki et al. (2011) who reported medullary bone P content of 16.87±0.34 in broiler breeder hens at 35 weeks of age. The current results are also in line with Chiripasi et al. (2013) who fed guinea fowl diet containing *I. belina* and found that inclusion of *I. belina* in guinea fowl diet had no influence on bone Ca contents of guinea fowl. The decrease in bone P and Ca contents with age in this study is consistent with Valable et al. (2018) who reported the decline of percentage bone P and Ca with increasing age of chickens. The reduction in Ca is expected, as the hen requires Ca and P for egg shell formation during the laying period. The mean meat P content of *Tswana* chickens reported in the current study is slightly higher (3.0±0.3 vs. 2.57± 0.19) than that reported by Mareko et al. (2010) on broiler chickens fed 40% level of *Phane* as supplement. The DM value (26.1±5.1) in the current study is consistent with De Marchi et al. (2005) who reported DM of 25.90±0.78 on breast of 25 weeks old female padovada breeds of chicken in Italy. The mean meat CP contents in the current study (76.6±7.7) compares very well with the findings of Saina (2005) (75.4% CP) on guinea fowl raised under an intensive rearing system and fed 18% CP, 13MJ/kg broiler phase 2 mash diet. Similar observations were made by Nsoso et al. (2008) (80.23±0.84%) on 5 weeks old keets raised on concrete floor and fed broiler starter diets in Botswana.

As illustrated in Figures 2 to 4, the taste panel scores for meat odour, flavour and juiciness did not show much differences across diets and ages. Odour and flavour received scores that averaged 4 (good odour and flavour) while juiciness received average score of 3 (moderate juiciness). This observation is supported by the earlier work of Mareko et al. (2006) who showed that guinea fowl meat had moderate to good odour. Mareko et al. (2010) reported that a higher percentage of panellists (41.67%) said that meat from broilers fed diets containing *I. belina* had a moderate odour. The present results are in agreement with Moreki et al. (2012) who fed guinea fowl diets containing maize, millet and sorghum as energy sources and reported that inclusion of cereal grain in guinea fowl diet did not influence organoleptic attributes of guinea fowl meat. It seems that feeding diets formulated with *I. belina* (Westwood), *V. subterranea* (L) Verde and *T. esculentum* (Burchell) Schreiber as sources of protein did not influence meat odour and flavour of *Tswana* hens. The observation that *Tswana* chickens meat received a moderate juicy rating in this study is in line with Mareko et al. (2006) who showed that over 66% of respondents felt that guinea fowl meat was moderately juicy when fed growers mash and reared on concrete and earth floors. Mareko et al. (2010) also reported moderate juiciness in broiler meat of chickens fed a diet containing *I. belina.* Similar findings were reported by Okeudo et al. (2006) in broiler chickens. Taste panel scores for odour intensity and tenderness in this study did not differ across diets and age. Odour intensity and tenderness received a moderate ranking that averaged 3 implying that diet supplemented with *I. belina* (Mopane worm) or *V. subterranea* (Bambara groundnut) or *T. esculentum* (Marama bean) did not affect odour intensity and tenderness of *Tswana* hens.

CONCLUSION

The bone P, Ca and K contents of *Tswana* hens raised under intensive system were not influenced by treatment diets. However, it was observed that these minerals decreased over time. Furthermore, dietary treatment had no influence on meat DM, CP, P, Ca and K of Tswana hens. Organoleptic properties of *Tswana* hens was not influenced by the incorporation of either *I. belina* or *V. subterranea* and also *T. esculentum i*n the layer diets as protein sources.

DECLARATIONS

Corresponding author
E-mail: fmanyeula@buan.ac.bw

Authors' contribution
All authors contributed equally to this work from starting proposal writing up to preparation of manuscript.

Competing Interests
The authors declare that they have no conflict of interest with regards to the research, authorship or publications of this manuscript.

Acknowledgements
The authors wish to acknowledge with gratitude the financial support provided by Useful Plants Project, Professor P.M. Kgwatalala for editing this work and Mr. J. Makore and Dr. B. Sebolai for assistance with statistics. Our special gratitude is also extended to Botswana University of Agriculture and Natural Resources for providing laboratory facilities and services.

To cite this paper: Manyeula F, Tsopito Ch-M, Mogotsi K, Nsoso Sh-J, Kamau JM and Moreki JC (2019). Chemical composition and sensory evaluation of meat of *Tswana* hens fed different protein sources: Mopane worm, Bambara groundnut and Morama bean. *Online J. Anim. Feed Res.*, 9(2): 92-102. www.ojafr.ir

REFERENCES

Aganga AA and Omphile CH (2000). Forage Resources of Botswana. Government Printers, Gaborone.

AOAC (2005). Association of Official Analytical Chemists. Official Methods of Analysis, 15th edition. Washington D.C.

Bower N, Hertel k, Oh J and Storey R (1988). Nutritional evaluation of Marama bean (Tylosema esculentum, Fabaceae): Analysis of the seed. Economic Botany, 42: 533–540. https://doi.org/10.1007/BF02862798

Belewu MA, Fagbemi T, Dosumu OO and Adeniyi MO (2008). Physicochemical and anti-nutritional properties of some lesser known tree and leguminous seeds. International Journal of Agricultural Research, 3: 237-242. DOI: 10.3923/ijar.2008.237.242

Chiripasi SC, Moreki JC, Nsoso SJ and Letso M (2013). Effects of feeding Mopane worm on mineral intake, retention and utilization in guinea fowl under intensive systems. International Journal of Poultry Science, 12: 19-28. DOI: 10.3923/ijps.2013.19.28.

De Marchi M, Cassandro M, Lunardi E, Baldan G and Siegel PB (2005). Carcass characteristics and qualitative meat traits of the Padovana breed of chicken. International Journal of Poultry Science, 4: 233-238. DOI: 10.3923/ijps.2005.233.238.

Dransfield E (1995). Tenderness of meat, poultry and fish, quality attributes and their measurement in meat, poultry and fish: Advanced Meat Research, 9: 288-310.

Seidler ES (2003). Egg Marketing. A Guide for the Production and Sale of Egg. FAO Agricultural Service Bulletin, Rome, Italy

Levie A (1979). Meat Handbook. 4th edition. Avi Publishing Company Inc., Westport, Connecticut.

Lewko L, Gornowicz E, Pietrzak M and Korol W (2017). The effects of origin, sex and feeding on sensory evaluation and some quality characteristics of goose meat from polish native flocks. Annual Animal Science, 17: 1185-1196. DOI: https://doi.org/10.1515/aoas-2017-0001.

Maltin C, Balcerzak D, Tilley R and Delday M (2003). Determinants of Eat Quality: Tenderness. Proceeding of the Nutrition Society, 62: 337-347.

Madibela OR, Mokwena KK, Nsoso SJ and Thema TF (2009). Chemical composition of Mopane worm sampled at three different sites in Botswana subjected to different processing. Tropical Animal and Health and Production, 41: 935-942. DOI: 10.1007/s11250-008-9282-7

Manyeula F, Tsopito CM, Kamau JM, Mogotsi KK, Nsoso SJ and Moreki JC (2018). Effects of *Imbrasia belina* (Westwood) or *Vigna subterranea* (L) Verde or *Tylosema esculentum* (Burchell) Schreiber as protein sources in diets fed to Tswana hens on egg quality. Journal of Animal Science and Veterinary Medicine, 3: 190-196. DOI: 10.31248/JASVM2018.118.

Mareko MDH, Nsoso SJ and Thibelang K (2006). Preliminary carcass and meat characteristics of guinea fowl (*Numidia meleagris*) raised on concrete and earth floors in Botswana. Journal of Food Science Technology, 4: 313-317. http://medwelljournals.com/abstract/?doi=jftech.2006.313.317.

Mareko MDH, Nsoso SJ, Mosweu K, Mokate KK and Madibela OR (2010). Chemical composition and sensory evaluation of broiler supplemented on two levels of Phane worm (*Imbrasia belina* (Westwood). Journal of Agricultural Applied Science, 6: 223-232.

Moreki JC, Podi KT, Nthoiwa PG and Machete JB (2012). Chemical analysis and sensory evaluation of guinea fowl meat fed diets containing three cereal grains as energy sources up to 12 weeks of age. International Journal of Science and Advanced. Technology, 2: 59-66.

Moreki JC, van der Merwe HJ and Hayes JP (2011). Effects of calcium level on egg production and shell quality in broiler breeder hens at peak production. Research Opinions in Animal and Veterinary Sciences. 1: 19-26. https://www.researchgate.net/publication/228506619.

NRC (1996). National Research Council. Nutrient Requirements of poultry, 9th Edition. National Academy Press., Washington D.C.

Nsoso SJ, Mareko MHD, Manyanda S and Legodimo PP (2008). The effects of housing type on body parameters feed intake and feed conversion ratio of guinea fowl (*Numidia meleagris*) keets and chemical composition of their meat during growth and development in Botswana. Research Journal of Animal Science, 2: 36-40.

Oduguwa OO, Oduguwa BO, Fanimo OA and Dipeolu MA (2000). Potency of two proprietary micronutrient premixes for broiler chickens at marginally deficient protein contents. Archivos de Zootecnia, 49: 433-444.

Okeudo NJ, Onyike IL, Okoli CV and Chielo IL (2006). Production performance, meat quality and feed cost implications of utilizing high levels of palm kernel cake in broiler finisher diets. International Journal of Poultry Science, 5: 1160-1163. DOI: 10.3923/ijps.2006.1160.1163.

Poste LM (1990). A sensory perspective of effects of feeds on flavour in meats: Poultry meats. Journal of Animal Science, 68: 4414-4420. DOI: 10.2527/1990.68124414x.

Rezaeipour V, Barsalani A and Abdullahpour R (2016). Effects of phytase supplementation on growth performance jejunum morphology liver health and serum metabolites of Japanese quails fed sesame (*Sesamum indicum*) meal-based diets containing graded levels of protein. Tropical Animal Health and Production 48: 1141-1146. DOI: 10.1007/s11250-016-1066-x.

Saina H (2005). Guinea Fowl (*Numidia meleagris*) production under smallholder farmer management in Guruve District, Zimbabwe. Masters of Philosophy Thesis. Department of Animal Science, Faculty of Agriculture, University of Zimbabwe. Doi: 10.1.1.533.4654&rep=rep1&type=pdf

SAS (2010). Statistical Analysis System, Users Guide Statistics. Version 9.3. Institute, Cary, NC, USA.

Sokołowicz Z, Krawczyk J and Świątkiewicz S (2016). Quality of poultry meat from native chicken breeds-A review. Annual Animal Science, 16: 347-368. DOI: 10.1515/aoas-2016-0004.

Valable AS, Narcy A, Duclos MJ, Pomar M, Page C, Nasir G and Létourneau-Montminy M (2018). Effects of dietary calcium and phosphorus deficiency and subsequent recovery on broiler chicken growth performance and bone characteristics. Animal, 12: 1555-1563. DOI: 10.1017/S1751731117003093.

To cite this paper: Manyeula F, Tsopito Ch-M, Mogotsi K, Nsoso Sh-J, Kamau JM and Moreki JC (2019). Chemical composition and sensory evaluation of meat of *Tswana* hens fed different protein sources: Mopane worm, Bambara groundnut and Morama bean. *Online J. Anim. Feed Res.*, 9(2): 92-102. www.ojafr.ir

2019 SCIENCELINE

Online Journal of Animal and Feed Research

Volume 9, Issue 2: 103-109; March 25, 2019

ISSN 2228-7701

COMPARISON OF FARM FORMULATED AND COMMERCIAL WINTER MINERAL LICK ON NUTRIENTS UTILIZATION AND SHEEP PERFORMANCE

Nchele KULEILE

Department of Animal Science, National University of Lesotho, P.O. Roma Lesotho

Supporting Information

ORIGINAL ARTICLE

PII: S222877011900013-9

Received: January 09, 2019

Accepted: February 17, 2019

Revised: March 25, 2019

ABSTRACT: A completely randomized study with three dietary treatments was undertaken at the National University of Lesotho Faculty of Agriculture farm. The three treatments were made up of control which had no access to winter mineral lick supplement and two treated groups made up of farm formulated and commercial winter lick. All animals had access to basal diet in the form of Teff [*Eragrostis tef* (Zucc.) Trotter]. The main objectives of the study were to assess the influence of supplementary feeding using winter mineral lick on sheep performance and on the utilization of teff. Also to compare both farm formulated and commercial winter mineral lick on the performance of sheep and utilization of *eragrostis tef*. A total of 30 yearling sheep of similar body weight were used in this study and were housed in pairs. Dietary treatments and water were offered ad libitum while basal diet was pre-weighed on daily basis. The findings of this study indicated that dietary treatments had a significant ($P<0.05$) influence on feed intake, live weight and weight gain while there were no significant ($P>0.05$) difference on feed conversion ratios. The control group of animals had poor production performance than treated groups and animals were losing weight. Nutrients digestibility were statistically different amongst all the treatments whereby supplement groups had better nutrient utilization than the control group. Mean comparison test revealed that there were no significant difference between farm formulated and commercial winter mineral lick on production and digestibility parameters. Economic benefit analysis indicated that by using farm formulated winter mineral lick farmers can save up to 36% of feed costs. To further reduce the costs of farm formulated lick, there is need to consider non-conventional feeds such as brewery by-products in the formulation of lick.

Keywords: Digestibility, *Eragrostis Tef*, Farm Formulated, Nutrient Utilization, Winter Mineral Lick

INTRODUCTION

A dry season in Lesotho is marked by periods of feed shortages resulting in general retardation in animal growth and production. Small ruminants diets in Lesotho are based on fibrous feeds: mainly mature unimproved pastures particularly at the end of the dry season and crop residues such as wheat straw, maize and sorghum stovers. These feeds are imbalanced particularly deficient in protein, minerals and vitamins and they are highly lignified with low digestibility (Habib et al., 2009; Khattab et al., 2013; Gado et al., 2016 and Sheikh et al., 2018). Not surprisingly the animals are malnourished to a great extent, especially during winter when the grazing land has limited quantities of forage. The lack of supplementary feeding practices by the farmers during this critical phase of production further exacerbated the low body condition scores and increased mortality of both lambs and their dams as well as increased reproductive failures.

In sheep, digestion is done with the help of rumen microbes particularly cellulolytic bacteria which convert structural carbohydrates into volatile fatty acids, carbonic acid and methane gas. Cellulolytic bacteria need a neutral pH between 6 and 9, while a pH less than 5.5 affects fiber digestibility (Castillo-González et al., 2014). The presence of extracellular cellulase enzymes is needed to break β-glycosidic bonds (1-4) of the biopolymer thereby providing sugars for use by microorganisms (Wedekind et al., 1988). Major microbial activities in the rumen involve multiplication and substrate degradation and the latter depends primarily on the availability of fermentable nitrogen, rumen un-degradable protein and readily available source of energy (Alvarez Almora et al., 2012). Factors that can lead to deficiencies of any of the three primaries can negatively affect digestion and digestibility of structural carbohydrates by the rumen microbes (Leng, 1991; Ushida and Jouany, 1990).

To cite this paper: Kuleile N and Molapo S (2019). Comparison of farm formulated and commercial winter mineral lick on nutrients utilization and sheep performance. *Online J. Anim. Feed Res.*, 9(2): 103-109. www.ojafr.ir

Urea is a form of non-protein nitrogen (NPN) and probably the most common source of fermentable nitrogen. Molassses on the other hand is a source of readily fermentable energy and it also acts as carrier of both urea and mineral supplements for ruminant animals (Hamed et al., 2012). Urea-molasses mineral blocks (UMMB) contains urea, molasses, salt, mineral mixture, bran and vitamins and can improve the utilization of low quality roughages by satisfying the requirements of the rumen microorganisms, creating a better environment for fermentation of fibrous material and increasing production of microbial protein and volatile fatty acids (Trishna et al., 2012). Urea after hydrolyzing into ammonia in the rumen provides a nitrogen source for rumen microflora which in turn improves the digestibility and utilization of fibrous feeds (Mengistu and Hassen, 2017; Azizi-Shotorkhoft et al., 2018 and Selthilkumar et al., 2018). Urea molasses supplement in sheep is effective in reducing the cost of supplementary feeds (Mubi et al., 2013).

The successful utilization of UMMB as supplement for small ruminants on production, digestibility and reproductive performance was reported by a number of researchers (Mubi et al., 2013; Hatungimana and Ndolisa, 2015; Gendley and Tiwari, 2016; Yatoo et al., 2016 and Mira et al., 2018). These researchers used UMMB as a supplement to improve utilization of poor quality basal diet and observed that UMMB had improved animal appetite, dry matter intake, milk production, body weight gain, feed conversion ratio, body condition score and digestibility. The aim of the current study was to assess the influence of UMMB as a supplement for merino sheep during a dry season and to compare the effect of commercial and farm formulated licks on production performance and nutrient utilization.

MATERIALS AND METHODS

Ethical Approval

The study met the animal welfare conditions standards for conducting animal science research set by the Faculty of Agriculture of the National University of Lesotho.

Study site

The study was conducted at the National University of Lesotho farm in Roma, some 34 kilometres southeast of Maseru, the capital of Lesotho. The Roma valley is broad and is surrounded by a barrier of rugged mountains which provide magnificent scenery. The climate of this area alternates between the hot and cold months. The winter being the coldest season and is experienced from May to July and temperature may drop as low as -1^{0}C, summer is the hottest season and is experienced from September to February and temperature can be as high as 28^{0}C.

Experimental design and treatments

The study was done using completely randomized design with three dietary treatments replicated five times. Dietary treatments were made up of control (sheep with no access to winter mineral lick), treatment one (sheep with access to commercial winter mineral lick) and treatment two (sheep with access to farm formulated winter mineral lick). Commercial winter mineral lick block was obtained from local animal feed supplier while farm formulated was mixed at the University of Lesotho farm using cold processing technique. Ingredients and their inclusion rates for farm formulated urea mineral block are shown below in Table 1.

Table 1 - Dietary ingredients and binding materials of winter mineral lick

Ingredients	Inclusion rate (%)
Molasses	35
Urea	15
Mineral mixture	2
Salt	5
Cement	13
Wheat bran	30
Total ingredients	100

Animal management

A total of (n=30) yearling male Merino sheep of similar live weight were used in this study where 10 sheep were allotted to each treatment. All animals were stall fed during the entire experimental period. Two sheep were housed in each pen which was cleaned regularly .The sheep were fed a pre- weighted basal diet daily (Table 2) made up of *Eragrostis teff*. Dietary treatments were offered ad libitum to sheep. Clean water was also given ad libitum. All routine management aspects for yearling sheep were observed. The feeding trial lasted for eight weeks including one week of adaptation period.

To cite this paper: Kuleile N and Molapo S (2019). Comparison of farm formulated and commercial winter mineral lick on nutrients utilization and sheep performance. *Online J. Anim. Feed Res.*, 9(2): 103-109. www.ojafr.ir

Table 2 - Basal diet (Eragrostis tef) chemical composition	
Constituents	**Percentages**
Dry matter	87.0
Ash	5.00
Crude Protein	8.50
Crude Fiber	2.20
Metabolisable energy (MJ/kgDM)	11.0
Ether Extract	2.20
Calcium	0.10
Phosphorus	0.30

Data collection

Data collection for this study was divided into three major areas namely production, digestibility and economic data.

Production data

Animal production data such as feed intake, live weight, weight gain and feed conversion ratio (FCR) were collected on weekly basis using the following formulas;

Feed intake = Total feed offered (kg) - Total feed refused (kg)

Feed Conversion Ratio = Feed Intake/Body Weight

Live Weight was determined by weighing the animals using Weigh Bridge

Weight gain was calculated as the difference in weekly live weight of the sheep

Digestibility data

Digestibility data was used as proxy for nutrient utilization by sheep. Data was collected for dry matter and protein digestibility every two weeks using total collection method for faeces. Digestibility was measured as the difference between feed intake and fecal output. Protein content was determined on both basal diet and small sample of faeces. Fecal samples for each pen was collected for a week, weighed and dried in the oven for 24 hours at 70^0C. A small sample of dried faeces was analyzed for protein content using kjeldahl procedure. The following formulas were used in the computation of dry matter and protein digestibility.

Dry matter digestibility = [(dry matter intake –fecal output) / dry matter intake] *100

Protein digestibility = [(protein intake – fecal output) / protein intake] *100

Economic benefits

The cost per kg of the experimental diet was calculated by multiplying the percentage composition of the ingredients with the price per kg of each feed ingredient and summing all the ingredients costs. Total feed intake x cost per kg feed gave total feed cost. Feed cost per kg weight gain was calculated as FCR x cost per kg of diet.

Data analysis

Data was analyzed with the aid of Studentized range Package for Social Sciences (IBM SPSS, 2011) version 20. Analyzes of variance (ANOVA) was used to determine the difference between the three dietary treatments. Least significant difference (LSD) at 5% was used to separate the means.

RESULTS AND DISCUSSIONS

Production parameters

The influence of winter mineral lick block on yearling sheep performance results are shown in Table 3. According to the results the dietary treatment had a significant ($P \leq 0.05$) influence on feed intake, live weight and daily weight gain whereby sheep that had access to winter mineral lick performed better than sheep without access to mineral lick. This increased *Eragrostis tef* intake by the treated group of animals is due to the resulting intense microbial activities due to increased nitrogen supply from the winter mineral lick. Feed conversion ratio results did not differ significantly ($P \geq 0.05$) between the three dietary treatments. The mean comparison test on the other hand revealed that there was not significant ($P \geq 0.05$) difference between sheep fed commercial and farm formulated winter mineral lick block. These results also highlighted the importance of supplementary feed because animals that were not given supplementary feeding (control group) had lower feed intake, live weight and daily weight gain compared to the supplemented groups. Sheep under control treatment were losing weight at the rate of 40g per head per day while animals receiving supplementary feeding were gaining an average weight of 38gram per head per day.

To cite this paper: Kuleile N and Molapo S (2019). Comparison of farm formulated and commercial winter mineral lick on nutrients utilization and sheep performance. *Online J. Anim. Feed Res.*, 9(2): 103-109. www.ojafr.ir

Data on weekly basis (Figures 1 to 2) also proved that animals that had access to winter mineral lick had higher feed intake and better live weight change across the entire experimental period. It was also observed from the current study that animals that did not have access to dietary treatment started losing weight linearly across all the weeks and this was also the similar case with feed intake which also declined linearly between week one and week four. These results indicated that the use of winter mineral lick had a significant influence on the utilization of *Eragrostis tef* whereby it increased the feed intake of treated group by 32% over the control group.

The findings of the current study are supported by the findings of Peterson et al. (1981); Tiwari et al. (1990); Nyarko et al. (1993); Tiwari et al. (2008); Chanjula and Ngampongsai (2008); Ali et al. (2009); Hatungimana and Ndolisha (2015); Mira et al. (2018) and Mengistu and Hassen (2018) who recorded an increase in dry matter intake by animals that received supplementary feeding in the form of winter mineral lick block than animals that were not offered supplementary feeds. The positive effect of winter mineral lick results on live weight maintenance and gain was confirmed by a number of researchers (Chen, 1993; Hadjipanayiotou et al., 1999; Zhang et al., 1999; Aganga et al., 2005; Mubi et al., 2012; Gendley and Tiwari, 2016; Yatoo et al., 2016; Baa et al., 2018 and Mengistu and Hassen, 2018) who found that animals that were supplemented with multi nutrients block significantly gained more weight than the control group which subsequently lost weight at the same time.

Table 3 - The effect of winter mineral lick on sheep performance

Parameters	Treatments			Significance	
	Control	Commercial	Own formulate	P[1]	CV[2]
Feed Intake(kg)	0.739[a]	1,047[b]	1,081[b]	0.001	3.29
Live weight (kg)	20.5[a]	22.5[b]	23.0[b]	0.001	6.34
FCR[3] (kg/kg)	1.26	1.49	1.64	0.200	3.95
Weight gain (kg)	-0.04[a]	0.038[b]	0.041[b]	0.001	2.44

a, b, Means in rows with different superscripts differ significantly (P<0.05); [1] Probability level at 0.05% [2] Coefficient of Variation; [3] Feed Conversion Ratio (gram feed/gram weight gain)

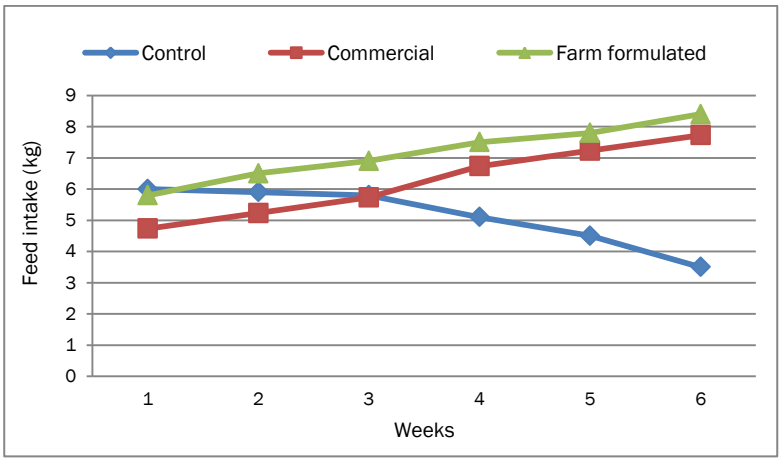


Figure 1 - Weekly feed intake

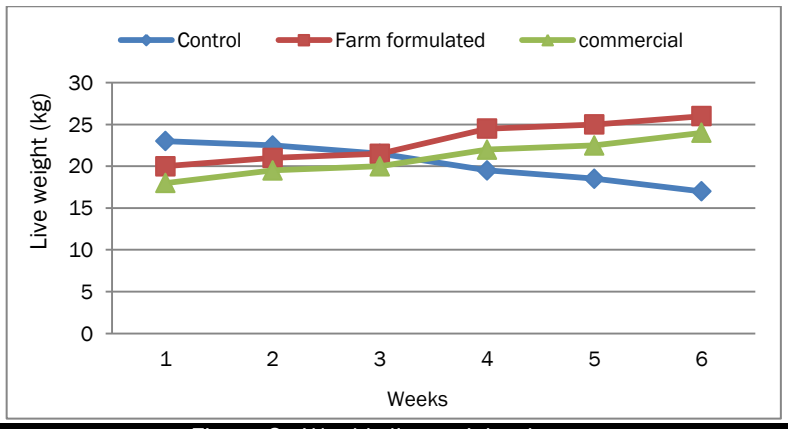


Figure 2 - Weekly live weight change

To cite this paper: Kuleile N and Molapo S (2019). Comparison of farm formulated and commercial winter mineral lick on nutrients utilization and sheep performance. *Online J. Anim. Feed Res.*, 9(2): 103-109. www.ojafr.ir

Nutrient digestibility

The comparison of farm formulated and commercial winter mineral lick on teff digestibility results are summarized in Table 4 below. The results showed that there were significant (P≤0.05) differences between treatment means in terms of *Eragrostis tef* dry matter and protein digestibility. The results also illustrated that there were significant (P≤0.05) difference between the control group and the two supplemented treatments in terms of teff digestibility and utilization whereby the control group had lower dry matter and protein digestibility. The mean comparison test results however, pointed out that there were no significant (P≥0.05) differences on teff dry matter and protein digestibility between both farm formulated and commercial winter mineral lick. The implication was that farm formulated winter mineral lick quality was good because it was able to give similar results to the commercial lick. The results on the influence of winter mineral lick supplementation on the utilization of teff highlighted that supplementary diet improved the utilization by thirty-two percent. The findings of the current study are in line with the findings of Garg and Gupta (1991); Khattab et al. (2013); Magalhaes et al. (2013) and Mengistu and Hassen (2018) who reported that supplementation of sheep with winter mineral lick improves the utilization and digestibility of low quality forages and maize stover. These results validated the theory that the use of winter mineral lick block improved the utilization and digestibility of low quality fodder in yearling sheep because the sheep that were not supplemented had poor utilization of *Eragrostis tef*.

Economic benefits

Economic benefits analysis results (Figure 3) indicated that the cost of 40kg commercial urea mineral lick was M255.00 while the cost for ingredients used in the formulation of own formulated urea mineral lick was M162.00. This implies that there was a reduction cost of 36% that was saved when using own formulated urea mineral lick as compare to buying commercial urea mineral lick.

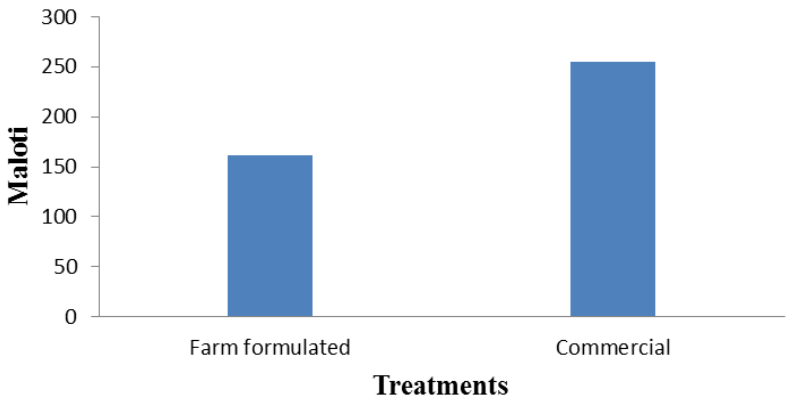


Figure 3 - The economic benefits of using farm formulated lick

Table 4 - The effect of winter mineral lick on *Eragrostis tef* nutrients digestibility

Parameters	Treatments			Significance	
	Control	Commercial	Own formulate	P[1]	CV[2]
Dry matter digestibility	54.07[a]	63.02[b]	63.09[b]	0.001	7.31
Protein digestibility	61.07[a]	70.02[b]	70.09[b]	0.001	6.55

[a, b] Means in rows with different superscripts differ significantly (P<0.05); [1] Probability level at 0.05%; [2] Coefficient of Variation

CONCLUSIONS

It can be concluded from the results that supplementary feeding especially during the dry season is very important for maintaining live weight of yearling sheep in Lesotho. The use of winter mineral lick block was able to maintain live weight, improved feed intake and utilization of *Eragrostis tef* and improved live weight gain by sheep. The adoption of formulating own mineral lick block also proved to be very important aspect that can help farmers to reduce their supplementary feeding by as much as thirty- six percent. The use of winter mineral lick block as a supplement also improved the digestibility and utilization of *Eragrostis tef* during the dry season by thirty-two percent which without the supplement (winter mineral lick block) would not be able to maintain live weight of yearling sheep.

To cite this paper: Kuleile N and Molapo S (2019). Comparison of farm formulated and commercial winter mineral lick on nutrients utilization and sheep performance. *Online J. Anim. Feed Res.*, 9(2): 103-109. www.ojafr.ir

DECLARATIONS

Corresponding Author
E-mail: Nchelekuleile@gmail.com

Authors' Contribution

NP Kuleile participated in the design of study, performed the experiments, analyzed the data, critically revised the manuscript for important intellectual contents, wrote the manuscript and approved the final manuscript.

Acknowledgements

The researcher would like to thank the National University of Lesotho, Faculty of Agriculture farm manager Mr. M. Mahlaha for his valuable time and advice during the implementation and the execution of the research trial. Mr. T. Jobo for proof reading and verification of references in the manuscript.

Conflict of interests

The authors have not declared any conflict of interests.

REFERENCES

Aganga AA, Lelata P and Tsiane MV (2005). Molasses urea blocks as supplementary feed resource for ruminants in Botswana. Journal of Animal and Veterinary Advances. 4(5): 524- 528. http://doi=javaa.2005.524.528

Ali I, Fontenot JP and Allen VG (2009). Palatability and drymatter intake by the sheep fed corn stover treated with different nitrogen sources. Pakistan Veterinary Journal.29 (4): 199-201 ISSN: 0253-8318.

Alvarez Almora EG, Huntington GB and Burns JC (2012). Effects of supplemental urea sources and feeding frequency on ruminal fermentation, fiber digestion, and nitrogen balance in beef steers. Animal Feed Science and Technology, 171:136-145. https://doi.org/10.1016/j.anifeedsci.2011.10.012

Azizi-Shotorkhoft A, Sharifi A, Azarfar A and Kiani A (2018). Effects of different carbohydrate sources on activity of rumen microbial enzymes and nitrogen retention in sheep fed diet containing recycled poultry bedding, Journal of Applied Animal Research, 46:1, 50-54, http:// doi:10.1080/09712119.2016.1258363

Baa A, Arbouche F, Arbouche R, Montaigne E, Arbouche Y and Arbouche HS (2018). Effects of incorporating oasis by-products on fattening performance and carcass characteristics of Ouled Djellal lamb, Veterinary World, 11(10): 1397-1403. http://doi:10.14202/vetworld.2018.1397-1403

Castillo-Gonzáleza AR, Burrola-Barrazab ME, Domínguez-Viverosb J, and Chávez-Martínezb A (2014). Rumen microorganisms and fermentation. Arch Med Vet 46, 349-361. http://dx.doi.org/10.4067/S0301-732X2014000300003

Chanjula P and Ngampongsai N (2008). Effect of supplemental nitrogen from urea on digestibility, rumen fermentation pattern, microbial populations and nitrogen balance in growing goats. Songklanakarin Journal of Science & Technology. 30 (5): 571-578. http://doi=10.1.1.626.6457&rep

Chen YZ, Wen H, Ma X, Li Y, Gao Z and Peterson MA (1993). Multinutrient lick blocks for dairy cattle in Gansu Province China. Livestock Research and Rural Development, 5(3):60-63.

Gado HM, Elghandour MMY, Cipriano M, Odongo NE and Salem AZM (2016). Rumen degradation and nutritive utilization of wheat straw, corn stalks and sugarcane bagasse ensiled with multienzymes. Journal of Applied Animal Research. 45 (1) 485-489. https://doi.org/10.1080/09712119.2016.1217866

Hamed K, Zary SY and Abdul WR (2012). The effect of molasses/mineral feed blocks and medicated blocks on performance, efficiency and carcass characteristics of Boer goat. Annals of Biological Research, 3(9): 4574-4577. ISSN 0976-1233

Hatungimana E and Ndolisa P (2015). Effects of urea molasses block supplementation on the growth performance of sheep. International Journal of Novel Research in Life Sciences 2:38-43. ISSN 2394-966X

IBM Corporation (2011). IMB SPSS Statistics for windows, Version 20.0. Armonk New York.

Gendley MK and Tiwari SP (2016). Effect of urea-molasses mineral block (UMMB) and medicated-UMMB supplementation on growth performance and feed gain ratio in goat kids. Sri Lanka Journal of Food and Agriculture. 2(1): 73-76. http://doi.org/10.4038/sljfa.v2i1.27

Khattab I, Salem A, Abdel-Wahed A and Kewan K(2013). Effect of urea supplementation on nutrient digestibility, nitrogen utilization and rumen fermentation in sheep fed diets containing dates. Livestock Science.155: 223-229. https://doi.org/10.1016/j.livsci.2013.05.024

Leng RA (1991). Application of biotechnology to nutrition of animals in developing countries. In: FAO animal production and health paper 90, Chapter 3. ISBN 92-5-103035-9.

Magalhães AF, Pires AJV, Carvalho GGP, da Silva FF, Filho CSN and Carvalho AO (2013).Intake, performance and nutrient digestibility of sheep fed sugarcane treated and ensiled with calcium oxide or urea. Revista Brasileira de Zootecnia. 42(10):691-699. ISSN 1806-9290

Mengistu G and Hassen W (2017). Review on: supplementary feeding of urea molasses multi-nutrient blocks to ruminant animals for improving productivity. International Journal of Animal Husbandry and Veterinary Science. 2(6) 43- 49. ISSN 2455-856

Mengistu G and Hassen W (2018). Supplementary feeding of urea molasses multi-nutrient blocks to ruminant animals for improving productivity. Academic Research Journal of Agricultural Science and Research. 6(2): 52-61.

https://doi: 10.14662/ARJASRD2017.062

Mubi AA, Mohammed ID and Kibon A (2013). Effects of multinutrient blocks supplementation on the performance of Yankasa sheep fed with basal diet of rice straw in the dry season of Guinea Savanna Region of Nigeria. Archives of Applied Science Research. 5 (4):172-178, ISSN 0975-508X

Nyarko-badohu DK, Kayouli C, Ba AA and Gasmi A (1993). Valorization of cereal straws in the feeding in the North of Tunisia. In Proceeding of the International Conference in Increasing Livestock Production through Utilization of Local resources Beijing, China. October 18–22–1993. Pp: 172–184.

Peterson JA, Klopsfentein TJ and Britton RA (1981). Ammonia treatment of corn plant residue: digestibility and growth rate. Journal of Animal Science. 53; 1524- 1592

Hadjipanayiotou ML, Verhaeghe AR, Kronfoleh LM, Labban M, Amin M, Al-Wadi A, Badran K, Dawa A, Shurbaji M, Houssein G, Malki T, Naigm A, Merawi R and Kader Harres A (1993). Urea blocks. II. Performance of cattle and sheep offered urea blocks in Syria. Livestock Research for Rural Development. 5(3).

Senthilkumar S, Sakthivel PC, Mahesh Kumar S, Sakthivadivu R, Bharathi N and Purushothaman MR (2018). Feeding strategy to improve the production performance of grazing sheep. International Journal of Science, Environment and Technology, 7(1): 278 – 283. ISSN: 2278-3687

Sheikh GG, Ganai AM, Reshi PA, Sheikh B and Shabir M (2018). Improved paddy straw as ruminant feed: A Review. Agricultural Research Communication Centre Journal, 39(2): 137-143. https:// doi:10.18805/ag.R-1667

Tiwari SP, Singh UB and Mishra UK (1990). Urea molasses mineral blocks as feed supplement: effect on growth and nutrient utilization in buffalo calves. Animal Feed Science & Technology, 29: 333–341.

Tiwari SP, Kumari K, Mishra UK, Gendley MK and Gupta R (2008). Effect of substituting concentrates mixture by urea molasses mineral block on protozoal production rates in Murrah buffalo caves. Livestock Research for Rural Development. 20 (11)

Trishna BK, Sanjeeb D and Rajeeb KR (2012).Impact of supplementation of UMMB licks in the ration of dairy animals. *Vetscan*, 7(1): 107-110. ISSN: 0973-6980

Ushida K and Jouany JP (1990). Effect of defaunation on fibre digestion in sheep given two isonitrogenous diets. Animal Feed Science and Technology. 29: 153-158. https://doi.org/10.1016/0377-8401(90)90101-D

Wedekind KJ, Mansfield HR and Montgomery L (1988). Enumeration and isolation of cellulolytic and hemicellulolytic bacteria from human feces. Applied Environmental Microbiology. 54: 1530-1535.

Yatoo MI, Kanwar MS and Ahmad MS (2016). Impact of area specific urea molasses mineral block on the production performance of Pashmina Goats. Advances in Animal and Veterinary Sciences. 4(6): 289 293.

http://dx.doi.org/10.14737/journal.aavs/2016/4.6.289.293

Zhang B, Li LL, Liu CY, Lin DM, Chen GW and Huang CL (1999). Effect of multinutrient lick blocks on performance of growing goats. Animal Ecology, 20(2): 4–8.

To cite this paper: Kuleile N and Molapo S (2019). Comparison of farm formulated and commercial winter mineral lick on nutrients utilization and sheep performance. *Online J. Anim. Feed Res.*, 9(2): 103-109. www.ojafr.ir

Instructions for Authors

OJAFR EndNote Style | Manuscript Template | Sample Articles | Declaration form

Manuscripts as Original Research Paper, Review, Short Communication and Case Reports and are invited for rapid peer-review publishing in the *Online Journal of Animal and Feed Research (ISSN 2228-7701)*.
Papers can be in any relevant fields of Animal Sciences (Animal Nutrition, Physiology, Reproduction, Genetics and Breeding, Behavior, Health, Husbandry and its economic, Animal products and Veterinary medicines of domestic animals) and relative topics. The journal does encourage papers with emphasis on the nutritive value and utilization of feeds that is depended to methods of Improvement, Assessment, Conserving and Processing feeds, Agronomic and climatic factors, Metabolic, Production, Reproduction and Health responses to dietary inputs (e.g., Feeds, Feed Additives, Specific Feed Components, Mycotoxins). Also, Mathematical models relating directly to animal-feed interactions, Analytical and experimental methods for Feed Evaluation as well as Animal Production studies with a focus on Animal Nutrition that do have link to a feed (Food Science and Technology) are acceptable relative topics for OJAFR**.**

Submission

The manuscripts should be submitted using our online submission system. For facile submission, please embed all figures and tables at the end of the manuscript to become one single file for submission. Once submission is complete, the system will generate a manuscript ID and password sent to author's contact email. If you have any difficulty in submitting the manuscript, kindly send via email: editors@ojafr.ir. All manuscripts must be checked (by English native speaker) and submitted in English for evaluation in totally confidential and impartial way.

Supplementary information:

Author guidelines are specific for each journal. Our MS Word template can assist you by modifying your page layout, text formatting, headings, title page, image placement, and citations/references such that they agree with the guidelines of journal. If you believe your article is fully edited per journal style, please use our Word template before submission. Supplementary materials may include figures, tables, methods, videos, and other materials. They are available online linked to the original published article. Supplementary tables and figures should be labeled with a "S", e.g. "Table S1" and "Figure S1". The maximum file size for supplementary materials is 10MB each. Please keep the files as small as possible to avoid the frustrations experienced by readers with downloading large files.

Submission to the Journal is on the understanding that:

1.The article has not been previously published in any other form and is not under consideration for publication elsewhere;
2.All authors have approved the submission and have obtained permission for publish work.
3.Researchers have proper regard for conservation and animal welfare considerations. Attention is drawn to the *'Guidelines for the Treatment of Animals in Research and Teaching'*. Any possible adverse consequences of the work for populations or individual organisms must be weighed against the possible gains in knowledge and its practical applications. If the approval of an ethics committee is required, please provide the name of the committee and the approval number obtained.

Ethics Committee Approval

Experimental research involving human or animals should have been approved by author's institutional review board or ethics committee. This information can be mentioned in the manuscript including the name of the board/committee that gave the approval. Investigations involving humans will have been performed in accordance with the principles of *Declaration of Helsinki*. And the use of animals in experiments will have observed the *Interdisciplinary Principles and Guidelines for the Use of Animals in Research, Testing, and Education* by the New York Academy of Sciences, Ad Hoc Animal Research Committee. If the manuscript contains photos or parts of photos of patients, informed consent from each patient should be obtained. Patient's identities and privacy should be carefully protected in the manuscript.

Presentation of the article

Main Format:

First page of the manuscripts must be properly identified by the title and the name(s) of the author(s). It should be typed in Times New Roman (font sizes: 12pt in capitalization for the title and the main text, double spaced, in A4 format with 2cm margins. All pages and lines of the main text should be numbered consecutively throughout the manuscript. The manuscript must be saved in a .doc format, (not .docx files). Abbreviations in the article title are not allowed except the well-known ones.

Manuscripts should be arranged in the following order:

a. TITLE (brief, attractive and targeted)
b. Name(s) and Affiliation(s) of author(s) (including post code) and corresponding E-mail
c. ABSTRACT
d. Key words (separate by semicolons; or comma,)
e. Abbreviations (used in the manuscript)
f. INTRODUCTION;
g. MATERIALS AND METHODS
h. RESULTS
i. DISCUSSION
j. CONCLUSION
k. DECLARATIONS
1. REFERENCES
m. Tables;
n. Figures

The sections "RESULTS AND DISCUSSION" can be presented jointly.
The sections "DISCUSSION AND DISCUSSION" can be presented jointly.

Article Sections Format:

Title should be a brief phrase describing the contents of the paper. Title Page should include full names and affiliations of the author(s), the name of the corresponding author along with phone and e-mail information. Present address(es) of author(s) should appear as a footnote.

Abstract should be informative and completely self-explanatory, briefly present the topic, state the scope of the experiments, indicate significant data, and point out major findings and conclusions. The abstract should be 150 to 300 words in length. Complete sentences, active verbs, and the third person should be used, and the abstract should be written in the past tense. Standard nomenclature should be used and abbreviations should be avoided. No literature should be cited.

Following the abstract, about 3 to 7 **key words** should be listed.

Introduction should provide a clear statement of the problem, the relevant literature on the subject, and the proposed approach or solution. It should be understandable to colleagues from a broad range of scientific disciplines.

Materials and Methods should be complete enough to allow experiments to be reproduced. However, only truly new procedures should be described in detail; previously published procedures should be cited, and important modifications of published procedures should be mentioned briefly. Capitalize trade names and include the manufacturer's name and address. Subheadings should be used. Methods in general use need not be described in detail.

Results should be presented with clarity and precision. The results should be written in the past tense when describing findings in the author(s)'s experiments. Previously published findings should be written in the present tense. Results should be explained, but largely without referring to the literature. Discussion, speculation and detailed interpretation of data should not be included in the results but should be put into the discussion section.

Discussion should interpret the findings in view of the results obtained in this and in past studies on this topic. State the conclusions in a few sentences at the end of the paper. The Results and Discussion sections can include subheadings, and when appropriate, both sections can be combined.

Conclusion should be brief and tight, providing a few specific tasks to accomplish: 1-Re-assert/Reinforce the Thesis; 2-Review the Main Points; 3- Close Effectively. The Conclusion section should not be similar to the Abstract content.

Declarations including Ethics, Consent to publish, Competing interests, Authors' contributions, and Availability of data and materials are necessary.

Acknowledgments of persons, grants, funds, etc should be brief.

Tables should be kept to a minimum and be designed to be as simple as possible. Tables are to be typed double-spaced throughout, including headings and footnotes. Each table should be on a separate page, numbered consecutively in Arabic numerals and supplied with a heading and a legend. Tables should be self-explanatory without reference to the text. The details of the methods used in the experiments should preferably be described in the legend instead of in the text. The same data should not be presented in both table and graph forms or repeated in the text.

The Figure legends should be typed in numerical order on a separate sheet. Graphics should be prepared using applications capable of generating high resolution GIF, TIFF, JPEG or PowerPoint before pasting in the Microsoft Word manuscript file. Use Arabic numerals to designate figures and upper case letters for their parts (Figure 1). Begin each legend with a title and include sufficient description so that the figure is understandable without reading the text of the manuscript. Information given in legends should not be repeated in the text.

DECLARATIONS

Please ensure that the sections: Ethics (and consent to participate), Consent to publish, Competing interests, Authors' contributions, and Availability of data and materials are included at the end of your manuscript in a Declarations section.

Consent to Publish

Please include a 'Consent for publication' section in your manuscript. If your manuscript contains any individual person's data in any form (including individual details, images or videos), consent to publish must be obtained from that person, or in the case of children, their parent or legal guardian. All presentations of case reports must have consent to publish. You can use your institutional consent form or our consent form if you prefer. You should not send the form to us on submission, but we may request to see a copy at any stage (including after publication). If your manuscript does not contain any individual person's data, please state "Not applicable" in this section.

Authors' Contributions

For manuscripts with more than one author, OJAFR require an Authors' Contributions section to be placed after the Competing Interests section. An 'author' is generally considered to be someone who has made substantive intellectual contributions to a published study. To qualify as an author one should 1) have made substantial contributions to conception and design, or acquisition of data, or analysis and interpretation of data; 2) have been involved in drafting the manuscript or revising it critically for important intellectual content; and 3) have given final approval of the version to be published. Each author should have participated sufficiently in the work to take public responsibility for appropriate portions of the content. Acquisition of funding, collection of data, or general supervision of the research group, alone, does not justify authorship.

We suggest the following format (please use initials to refer to each author's contribution): AB carried out the molecular genetic studies, participated in the sequence alignment and drafted the manuscript. JY carried out the immunoassays. MT participated in the sequence alignment. ES participated in the design of the study and performed the statistical analysis. FG conceived of the study, and participated in its design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript. For authors that equally participated in a study please write '**All/Both authors contributed equally to this work**.' Contributors who do not meet the criteria for authorship should be listed in an acknowledgements section.

Competing Interests

Competing interests that might interfere with the objective presentation of the research findings contained in the manuscript should be declared in a paragraph heading "Competing interests" (after Acknowledgment section and before References). Examples of competing interests are ownership of stock in a company, commercial grants, board membership, etc. If there is no competing interest, please use the statement "The authors declare that they have no competing interests.". *Online Journal of Animal and Feed Research* adheres to the definition of authorship set up by the International Committee of Medical Journal Editors (ICMJE). According to the ICMJE authorship criteria should be based on 1) substantial contributions to conception and design of, or acquisition of data or analysis and interpretation of data, 2) drafting the article or revising it critically for important intellectual content and 3) final approval of the version to be published. Authors should meet conditions 1, 2 and 3. It is a requirement that all authors have been accredited as appropriate upon submission of the manuscript. Contributors who do not qualify as authors should be mentioned under Acknowledgements.

Change in authorship

We do not allow any change in authorship after provisional acceptance. We cannot allow any addition, deletion or change in sequence of author name. We have this policy to prevent the fraud.

Acknowledgements

We strongly encourage you to include an Acknowledgements section between the Authors' contributions section and Reference list. Please acknowledge anyone who contributed towards the study by making substantial contributions to conception, design,

acquisition of data, or analysis and interpretation of data, or who was involved in drafting the manuscript or revising it critically for important intellectual content, but who does not meet the criteria for authorship. Please also include their source(s) of funding. Please also acknowledge anyone who contributed materials essential for the study.
Authors should obtain permission to acknowledge from all those mentioned in the Acknowledgements. Please list the source(s) of funding for the study, for each author, and for the manuscript preparation in the acknowledgements section. Authors must describe the role of the funding body, if any, in study design; in the collection, analysis, and interpretation of data; in the writing of the manuscript; and in the decision to submit the manuscript for publication.

Data Deposition

Nucleic acid sequences, protein sequences, and atomic coordinates should be deposited in an appropriate database in time for the accession number to be included in the published article. In computational studies where the sequence information is unacceptable for inclusion in databases because of lack of experimental validation, the sequences must be published as an additional file with the article.

References

An OJAFR reference style for **EndNote** may be found **here**.

1. All references to publications made in the text should be presented in a list with their full bibliographical description.
2. In the text, a reference identified by means of an author‘s name should be followed by the date of the reference in parentheses. When there are more than two authors, only the first author‘s surname should be mentioned, followed by ’et al‘. In the event that an author cited has had two or more works published during the same year, the reference, both in the text and in the reference list, should be identified by a lower case letter like ’a‘ and ’b‘ after the date to distinguish the works.
3. References in the text should be arranged chronologically (e.g. Kelebeni, 1983; Usman and Smith, 1992 and Agindotan et al., 2003). 'et al.' should not be italic. The list of references should be arranged alphabetically on author's surnames, and chronologically per author. If an author's name in the list is also mentioned with co-authors, the following order should be used: Publications of the single author, arranged according to publication dates - publications of the same author with one co-author - publications of the author with more than one co-author. Publications by the same author(s) in the same year should be listed as 1992a, l992b, etc.
4. Names of authors and title of journals, published in non-Latin alphabets should be transliterated in English.
5. A sample of standard reference is "1th Author surname A, 2th Author surname B and 3th Author surname C (2013). Article title should be regular, in sentence case form, and 9 pt. Online Journal of Animal and Feed Research, Volume No. (Issue No.): 00-00." (Journal titles should be full and not italic.)
6. If available please add DOI numbers or the link of articles at the end of each reference.

Examples (at the text):

Abayomi (2000), Agindotan et al. (2003), (Kelebeni, 1983), (Usman and Smith, 1992), (Chege, 1998; Chukwura, 1987a,b; Tijani, 1993,1995), (Kumasi et al., 2001).

Examples (at References section):

a) For journal:

Lucy MC (2000). Regulation of ovarian follicular growth by somatotropin and insulin- like growth factors in cattle. Journal of Dairy Science, 83: 1635-1647. Link, DOI

Kareem SK (2001). Response of albino rats to dietary level of mango cake. Journal of Agricultural Research and Development, Pp. 31-38. Link, DOI

Chikere CB, Omoni VT and Chikere BO (2008). Distribution of potential nosocomial pathogens in a hospital environment. African Journal of Biotechnology, 7: 3535-3539. Link, DOI

b) For symposia reports and abstracts:

Cruz EM, Almatar S, Aludul EK and Al-Yaqout A (2000). Preliminary Studies on the Performance and Feeding Behaviour of Silver Pomfret (Pampus argentens euphrasen) Fingerlings fed with Commercial Feed and Reared in Fibreglass Tanks. Asian Fisheries Society Manila, Philippine, 13: 191-199. Link, DOI

c) For edited symposia, special issues, etc., published in a journal:

Korevaar H (1992). The nitrogen balance on intensive Dutch dairy farms: a review. In: A. A. Jongebreur et al. (Editors), Effects of Cattle and Pig Production Systems on the Environment: Livestock Production Science, 31: 17-27. Link, DOI

d) For books:

AOAC (1990). Association of Official Analytical Chemists. Official Methods of Analysis, 15th Edition. Washington D.C. pp. 69-88. Pelczar JR, Harley JP, Klein DA (1993). Microbiology: Concepts and Applications. McGraw-Hill Inc., New York, pp. 591-603. Link, DOI

e) Books, containing sections written by different authors:

Kunev M (1979). Pig Fattening. In: A. Alexiev (Editor), Farm Animal Feeding. Vol. III. Feeding of Different Animal Species, Zemizdat, Sofia, p. 233-243 (Bg). Link, DOI

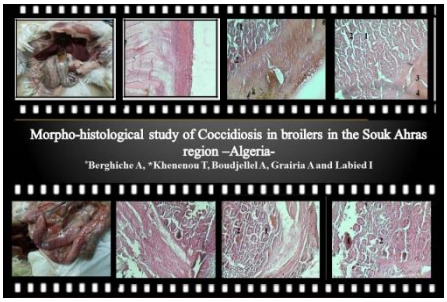
In referring to a personal communication the two words are followed by the year, e.g. (Brown, J. M., personal communication, 1982). In this case initials are given in the text. Where available, URLs for the references should be provided.

Formulae, numbers and symbols

1. Typewritten formulae are preferred. Subscripts and superscripts are important. Check disparities between zero (0) and the letter 0, and between one (1) and the letter I.
2. Describe all symbols immediately after the equation in which they are first used.
3. For simple fractions, use the solidus (/), e.g. 10 /38.
4. Equations should be presented into parentheses on the right-hand side, in tandem.
5. Levels of statistical significance which can be used without further explanations are *P < 0.05, **P < 0.01, and ***P<0.001.
6. In the English articles, a decimal point should be used instead of a decimal comma.
7. Use Symbol fonts for "±"; "≤" and "≥" (avoid underline).
8. In chemical formulae, valence of ions should be given, e.g. Ca2+ and CO32-, not as Ca++ or CO3.
9. Numbers up to 10 should be written in the text by words. Numbers above 1000 are recommended to be given as 10 powered x.
10. Greek letters should be explained in the margins with their names as follows: Aα - alpha, Bβ - beta, Γγ - gamma, Δδ - delta, Eε - epsilon, Zζ - zeta, Hη - eta, Θθ - theta, Iι - iota, Kκ - kappa, Λλ - lambda, Mμ - mu, Nν - nu, Ξξ - xi, Oo - omicron, Ππ - pi, Pρ - rho, Σσ - sigma, Tτ - tau, Yυ - ipsilon, Φφ - phi, Χχ - chi, Ψψ - psi, Ωω - omega. Please avoid using math equations in Word whenever possible, as they have to be replaced by images in xml full text.

Abbreviations
Abbreviations should be presented in one paragraph, in the format: "term: definition". Please separate the items by ";".
E.g. ANN: artificial neural network; CFS: closed form solution; ....

Graphical Abstract:
Authors of accepted articles should provide a graphical abstract (a beautifully designed feature figure) to represent the paper aiming to catch the attention and interest of readers. Graphical abstract will be published online in the table of content. The graphical abstract should be colored, and kept within an area of 12 cm (width) x 6 cm (height) or with similar format. Image should have a minimum resolution of 300 dpi and line art 1200dpi.
Note: Height of the image should be no more than the width. Please avoid putting too much information into the graphical abstract as it occupies only a small space. Authors can provide the graphical abstract in the format of PDF, Word, PowerPoint, jpg, or png, after a manuscript is accepted for publication.
If you have decided to provide a Professional Graphical Abstract, please click **here**.



Review/Decisions/Processing

Firstly, all manuscripts will be checked by Docol©c, a plagiarism finding tool. The received papers with plagiarism rate of more than 40% will be rejected. Manuscripts that are judged to be of insufficient quality or unlikely to be competitive enough for publication will be returned to the authors at the initial stage. The remaining manuscripts go through a double-blind review process by two reviewers selected by section editor (SE) or deputy SE of OJAFR, who are research workers specializing in the relevant field of study. One unfavorable review means that the paper will not be published and possible decisions are: accept as is, minor revision, major revision, or reject. The corresponding authors should submit back their revisions within 14 days in the case of minor revision, or 30 days in the case of major revision.

To submit a revision please click here, fill out the form, and mark "Revised", mention the article code (for example OJAFR-1108), attach the revision (MS word) and continue submission. Manuscripts with significant results are typically reviewed and published at the highest priority. After review and editing the article, a final formatted proof is sent to the corresponding author once again to apply all suggested corrections during the article process. The editor who received the final revisions from the corresponding authors shall not be hold responsible for any mistakes shown in the final publication.

The submissions will be processed free of charge for invited authors, authors of hot papers, and corresponding authors who are editorial board members of the *Online Journal of Animal and Feed Research*. This journal encourages the academic institutions in low-income countries to publish high quality scientific results, free of charges.

Plagiarism: There is an instant policy towards plagiarism (including self-plagiarism) in our journals. Manuscripts are screened for plagiarism by Docol©c, before or during publication, and if found they will be rejected at any stage of processing.

Date of issue
All accepted articles are published bimonthly around 25th of January, March, May, July, September and November, each year in full text on the Internet.

The OA policy
Online Journal of Animal and Feed Research is an Open Access journal which means that all content is freely available without charge to the user or his/her institution. Users are allowed to read, download, copy, distribute, print, search, or link to the full texts of the articles, or use them for any other lawful purpose, without asking prior permission from the publisher or the author. This is in accordance with the *BOAI definition of Open Access*.

Submission Preparation Checklist
Authors are required to check off their submission's compliance with all of the following items, and submissions may be returned to authors that do not adhere to the following guidelines:
- The submission has not been previously published, nor is it before another journal for consideration (or an explanation has been provided in -Comments to the Editor).
- The submission file is in Microsoft Word, RTF, or PDF document file format.
- Where available, URLs for the references have been provided.
- The text is double-spaced; uses a 12-point font; and all illustrations, figures, and tables are placed within the text at the appropriate points, rather than at the end.
- The text adheres to the stylistic and bibliographic requirements outlined in the Author Guidelines.

Editorial Offices:
Atatürk University, Erzurum 25100, Turkey
University of Manitoba, Winnipeg, Manitoba R3T 2N2, Canada
University of Maragheh, East Azerbaijan, Maragheh 55136, Iran
Homepage: www.science-line.com
Phone: +98 914 420 7713 (Iran); +90 538 770 8824 (Turkey); +1 204 8982464 (Canada)
Emails: administrator@science-line.com ; saeid.azar@atauni.edu.tr

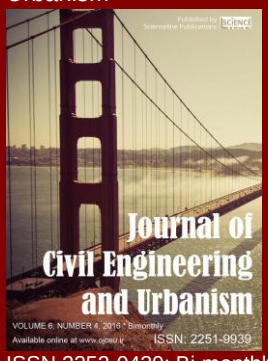
SCIENCELINE PUBLISHING CORPORATION

Scienceline Publication Ltd is a limited liability non-profit non-stock corporation incorporated in Turkey, and also is registered in Iran. Scienceline journals that concurrently belong to many societies, universities and research institutes, publishes internationally peer-reviewed open access articles and believe in sharing of new scientific knowledge and vital research in the fields of life and natural sciences, animal sciences, engineering, art, linguistic, management, social and economic sciences all over the world. Scienceline journals include:

Online Journal of Animal and Feed Research

ISSN 2228-7701; Bi-monthly
View Journal I Editorial Board
Email: editors@ojafr.ir
Submit Online >>

Journal of Civil Engineering and Urbanism



ISSN 2252-0430; Bi-monthly
View Journal I Editorial Board
Email: ojceu@ojceu.ir
Submit Online >>

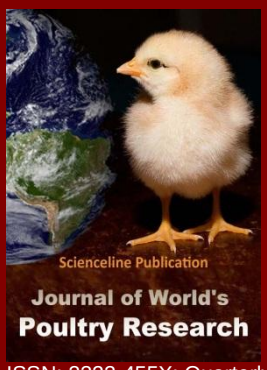
Journal of Life Sciences and Biomedicine

ISSN: 2251-9939; Bi-monthly
View Journal I Editorial Board
Email: editors@jlsb.science-line.com
Submit Online >>

Asian Journal of Medical and Pharmaceutical Researches

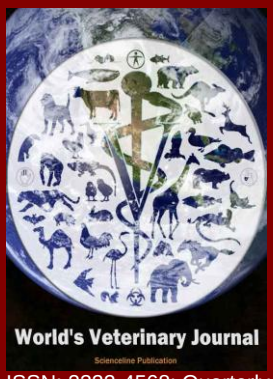
ISSN: 2322-4789; Quarterly
View Journal I Editorial Board
Email: editor@ajmpr.science-line.com
Submit Online >>

Journal of World's Poultry Research



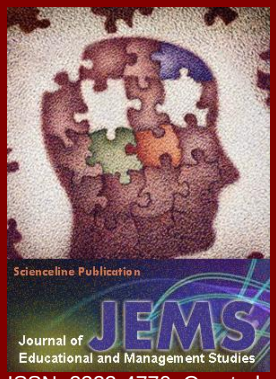
ISSN: 2322-455X; Quarterly
View Journal I Editorial Board
Email: editor@jwpr.science-line.com
Submit Online >>

World's Veterinary Journal



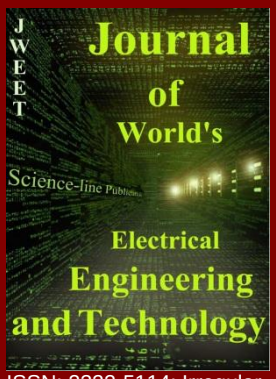
ISSN: 2322-4568; Quarterly
View Journal I Editorial Board
Email: editor@wvj.science-line.com
Submit Online >>

Journal of Educational and Management Studies



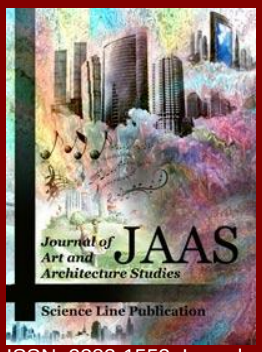
ISSN: 2322-4770; Quarterly
View Journal I Editorial Board
Email: info@jems.science-line.com
Submit Online >>

Journal of World's Electrical Engineering and Technology



ISSN: 2322-5114; Irregular
View Journal I Editorial Board
Email: editor@jweet.science-line.com
Submit Online >>

Journal of Art and Architecture Studies



ISSN: 2383-1553; Irregular
View Journal I Editorial Board
Email: jaas@science-line.com
Submit Online >>

Asian Journal of Social and Economic Sciences

ISSN: 2383-0948; Quarterly
View Journal I Editorial Board
Email: ajses@science-line.com
Submit Online >>

Journal of Applied Business and Finance Researches

ISSN: 2382-9907; Quarterly
View Journal I Editorial Board
Email: jabfr@science-line.com
Submit Online >>

Scientific Journal of Mechanical and Industrial Engineering

ISSN: 2383-0980; Quarterly
View Journal I Editorial Board
Email: sjmie@science-line.com
Submit Online >>