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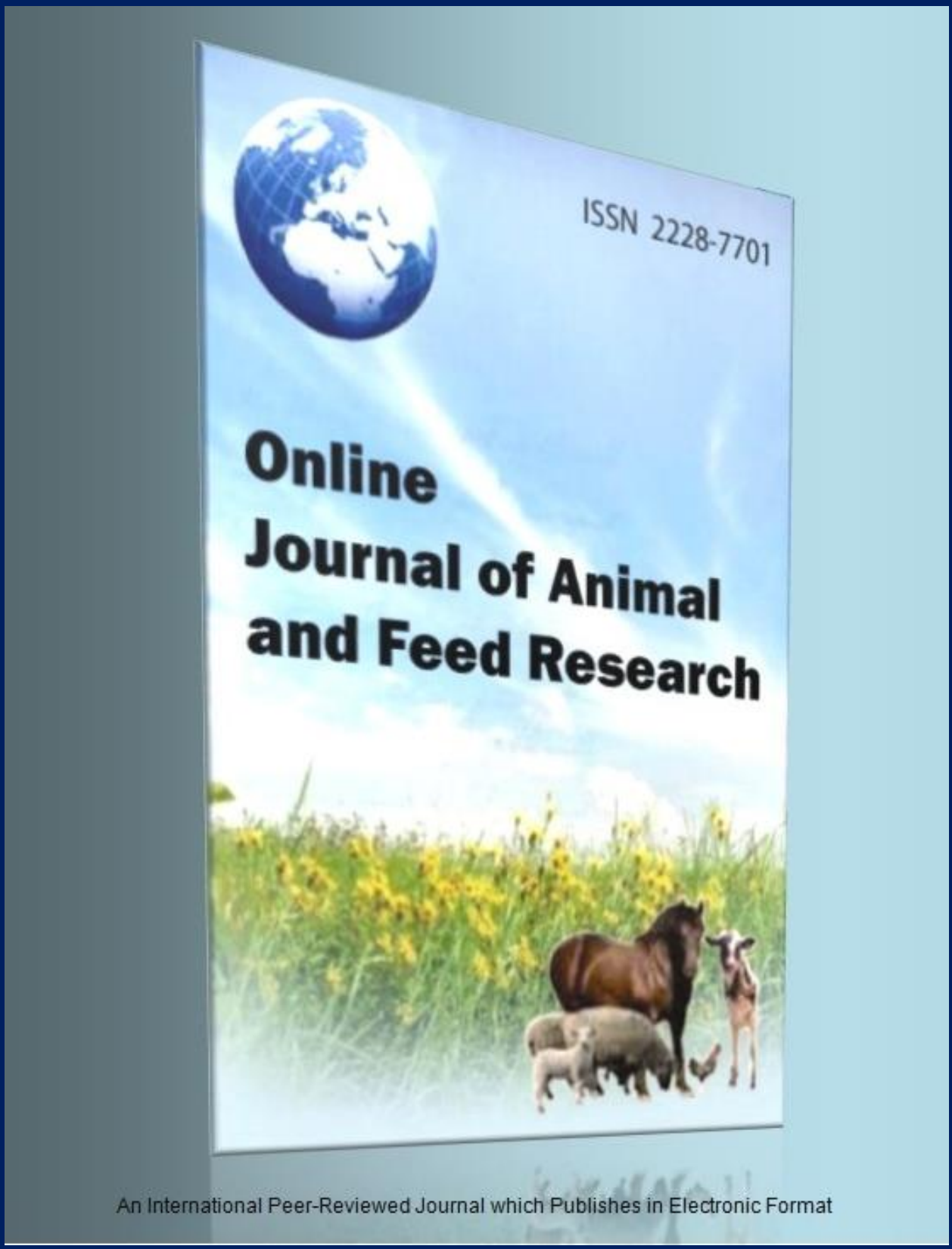

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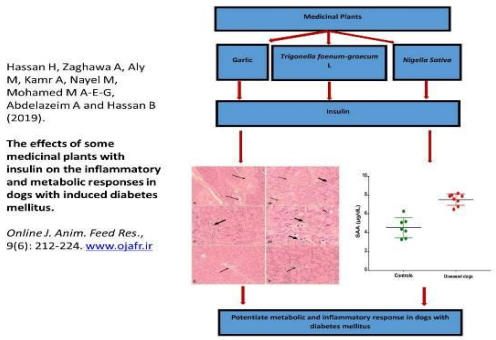
Volume 9 (6); November 25, 2019

Review

The effects of some medicinal plants with insulin on the inflammatory and metabolic responses in dogs with induced diabetes mellitus.

Hassan H, Zaghawa A, Aly M, Kamr A, Nayel M, Mohamed M A-E-G, Abdelazeim A and Hassan B.

Online J. Anim. Feed Res., 9(6): 212-224, 2019; pii: S222877011900030-9



Abstract
The goals of this study were to determine biochemical biomarkers and histopathological changes in addition to exploring insulin as mono-therapy as well as insulin plus some medical plants include Garlic, Fenugreek (*Trigonella foenum-graecum* L.) and Black seeds (*Nigella Sativa*) as feed additives in dogs with experimentally induced diabetes mellitus (DM). A total of 15 clinically apparent healthy adult dogs were involved in this study and divided into group 1: included five normal adult dogs served as non-diabetic control group, group 2: Included five adult dogs that were subjected to experimentally diabetes mellitus by intravenous (IV) injection of alloxan and treated by with insulin only and group 3: Included five adult dogs that were subjected to experimentally diabetes mellitus by IV injection of alloxan and treated by with insulin plus once daily supplement with herbal therapy. In conclusions, the therapeutic effects of some medical plants include Garlic, Fenugreek (*Trigonella foenum-graecum* L.) and Black seeds (*Nigella Sativa*) increased the potency and glycemic control of insulin in animals with diabetes mellitus. Therefore, it is recommended adding the previously mentioned herbs as feed additives to improve the potency of insulin to control DM in dogs.

Keywords: Dogs; Diabetes mellitus, Alloxan hydrate; Insulin; Insulin with herbal therapy

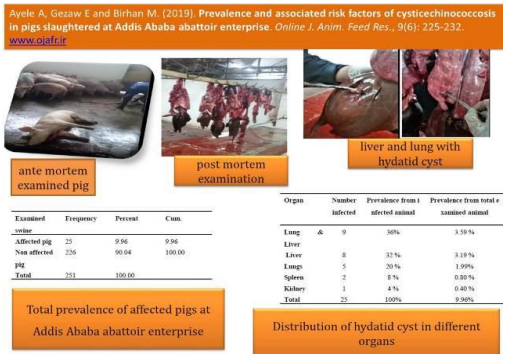
[Full text-PDF]

Research Paper

Prevalence and associated risk factors of cysticechinococcosis in pigs slaughtered at Addis Ababa abattoir enterprise.

Ayele A, Gezaw E and Birhan M.

Online J. Anim. Feed Res., 9(6): 225-232, 2019; pii: S222877011900031-9



Abstract
A cross sectional study was conducted from January 2018 to April 2018 to determine the prevalence and associated risk factors of cystic echinococcosis in pig slaughtered at Addis Ababa abattoir enterprise, central part of Ethiopia. A total of 251 pigs were randomly sampled and routine meat inspection procedure was employed to detect the presence of hydatid cyst in the visceral organs (lung, liver, spleen and kidney), where 25 (9.96%) pigs were positive. Analysis of risk factors for occurrence of the disease revealed that there was statistically significant variation ($P < 0.05$) in swine with different body condition scores and age groups. However, significant variation was not observed ($P > 0.05$) across different sex and origin. Prevalence of distribution of hydatid cyst in different organs from total examined swine were 3.59%, 3.19%, 1.99%, 0.80%, 0.40%, for lungs and livers, livers, lungs, spleens and kidney respectively. In this study, the liver was found to be the most predominantly affected organ (6.77%) followed by the lungs (5.58%), spleen (0.80%) and the least affected organ was kidney (0.40%). As regards size of the cyst from total infected organs, organ with small sized cysts had the highest percentage (67.6%), followed by medium sized cysts (20.6%) and large sized cysts (11.8%). Livers (44.1%) were predominant organ infected with small cyst size while spleen (5.9%) and kidney (2.9%) have only small cyst size. Lungs (14.7%) and liver (5.9%) were infected with medium cyst size while only lungs (11.8%) have large cyst size. From the total of 34 (100%) affected organs, only 4 (11.8%) lungs have more than or equal to three cyst numbers while remaining 30 (88.2 %) affected organs were with less than three cyst numbers.

Keywords: Cyst number, Cyst size, Echinococcosis, Echinococcus granulosus, Hydatid cysts, Prevalence, Risk factors.

[Full text-PDF]

Review

Non-surgical castration methods to control stray dog population, a review.

Seid AM and Terefe DA (2019). **Non-surgical castration methods to control stray dog population, a review.** *Online J. Anim. Feed Res.*, 9(6): 233-240. www.ojafr.ir

Seid AM and Terefe DA.
Online J. Anim. Feed Res., 9(6): 233-240, 2019; pii: S222877011900032-9

Abstract
About 75% of dogs worldwide are free to roam and reproduce, thus creating locally overabundant populations. Problems caused by roaming dogs include diseases transmission to livestock and humans, predation on livestock, attacks on humans, road traffic accidents, and nuisance behavior. Nonsurgical fertility control is increasingly advocated as more cost-effective than surgical sterilization to manage dog populations and their impact. The aim of this review was to illustrate the spectrum of fertility inhibitors available for dogs. Although surgery is the most effective and safe procedure, it is also expensive to use of non-surgical, sterilization methods that would make male sterilization inexpensive, easy and fast for sterilization of large number of male dogs within short period of time to effectively contribute in curbing the growth of the stray dog population were introduced. Chemical sterilization methods so far employed included hormonal methods, immunocontraceptives and ınorganic chemo-sterilants (chemo-sterilants such as $CaCl_2$, zinc gluconate neutralized by arginine (Neutersol) and hypertonic sodium chloride-NaCl solution. Intratesticular injection of calcium chloride, Zinc gluconate and 20% NaCl hypertonic solution showed a promising result as chemical sterilants. The review concluded that the main challenges for the future are evaluating the feasibility, effectiveness, sustainability, and effects of mass non-surgical sterilization campaigns on dog population size and impact as well as integrating nonsurgical fertility control with disease vaccination and public education programs. The review also showed the relative lack of research or knowledge related to fertility inhibitors in developing country as Ethiopia and suggested that more works is required in this country.

Keywords: Chemical sterilization, Dog population management, Fertility inhibitors, Stray Dogs.

[Full text-PDF]

Research Paper

Levels of aflatoxins and fumonisins in poultry feed from Ghana.

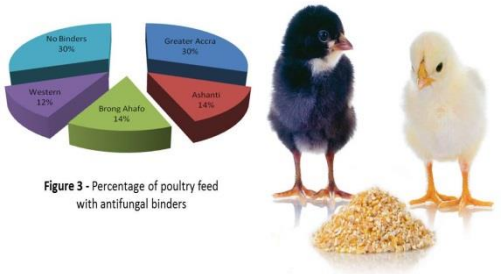


Figure 3 - Percentage of poultry feed with antifungal binders

Kumi J and Agyei-Heneku KA A-O (2019). Levels of aflatoxins and fumonisins in poultry feed from Ghana. *Online J. Anim. Feed Res.*, 9(6): 241-246. www.ojafr.ir

Kumi J and Agyei-Heneku KA A-O.
Online J. Anim. Feed Res., 9(6): 241-246, 2019; pii: S222877011800033-9

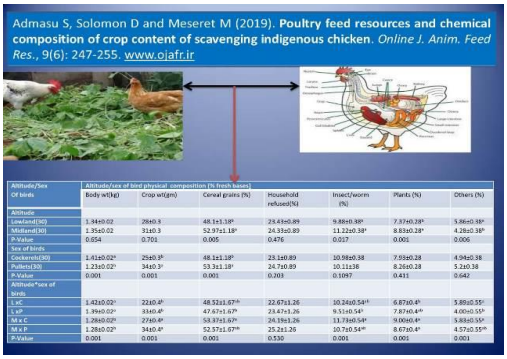
Abstract
Mycotoxins are secondary fungal metabolites that contaminate animal feeds, crops and food. Globally, two major foodborne mycotoxins (aflatoxins and fumonisins) have been reported to affect the health and productivity of the poultry industry. Notwithstanding the health risks associated with these mycotoxins, no study has probably investigated the co-occurrence of aflatoxin and fumonisin levels in poultry feed produced in Ghana. The aim of the study was to investigate the levels of total aflatoxin (B1, B2, and G1 and G2) and fumonisin B1 (FB_1) levels in poultry feed produced in Ghana. Total aflatoxins and fumonisin B1 were analyzed in 100 poultry feed samples collected from farmers from four major poultry producing regions in Ghana. High performance liquid chromatography (HPLC) was used to measure total aflatoxin levels whiles a fluorometer reader was used to measure the levels of fumonisin B1. Total aflatoxin and FB1 contaminations were detected in 100% of the feed samples in a range of 0.02-22 ppb and 0.5-4.6 ppm, respectively. Three samples representing, 3% out of the 100 samples screened were detected to have total aflatoxin levels greater than 20 parts per billion Fumonisin levels detected in the poultry feed were within the permissible level of 50 parts per million in poultry feed. Seventy out of the 100 poultry feed samples collected had their feed treated with fungal binders. In present study, we have probably for the first time in Ghana shown the levels of mycotoxins in poultry feed and the need to monitor animal feed made from cereals.

Keywords: Poultry feed, Total aflatoxin, Fumonisin B1, Ghana

[Full text-PDF]

Research Paper

Poultry feed resources and chemical composition of crop content of scavenging indigenous chicken.



Admasu S., Solomon D. and Meseret M.
Online J. Anim. Feed Res., 9(6): 247-255, 2019; pii: S222877011800034-9

Abstract
The study was conducted in Genji district of West Wollega Zone with the objectives of characterization of scavenging poultry feed resource base (SFRB) and evaluation of composition of crop content of scavenging indigenous chicken. A total of 60 sampled grower chickens (50% female and 50% male) at an age of 4-6 month, were purchased fromrural farmers and slaughtered during early dry season to study the physical characteristics and chemical composition of the crop content. About 50.7, 23.85, 12, 8.4 and 5.2% of the crop contents of experimental chickens were cereal grains, house-hold leftover/kitchen waste, animal proteins (insects/worms), plant/leaves, and none feed materials respectively. There was variation in composition with altitude and sex of birds slaughtered. The mean weight of the crop content obtained from the cockerels (25 g/day) was significantly lower ($P < 0.05$) than that of the pullets (34 g/day, but there were no significant difference between altitude in mean weight of crop content of the experimental birds slaughtered. According to the result of laboratory analysis, the dry matter, ether extract, ash, crude protein, crude fiber, nitrogen free extract and calculated metabolizable energy content of the crop content were 89.37, 2.48, 14.82, 10.88, 9.35, 62.61% and 2552.3 kcal/kg), respectively. The percent composition of dry matter, ash, crude fiber, and calcium were significantly ($P < 0.05$) higher in the crop content of pullets than in the crop content of cockerels, while crude fiber and crude protein level of the crop contents of the chickens of the mid altitude were significantly higher ($P < 0.05$) than that of the crop content of chickens of the low altitude. The study showed that the nutrient contents of Scavengable feed resources were below the bird's requirements for optimum productivity. In conclusion, Poultry keepers must provide sufficient supplementation to their birds rather than simply throwing leftovers away to the birds.

Keywords: Chemical Composition, Crop Content, Indigenous Chicken, Scavengable Feed Resources.

[Full text-PDF]

Research Paper

Prevalence and economic significance of bovine fasciolosis in cattle slaughtered at Debre-Tabor municipal abattoir.

Birhan M and Demewez G, Tewodros F, and Tadegenge M (2019). **Prevalence and economic significance of bovine fasciolosis in cattle slaughtered at Debre-Tabor municipal abattoir.** *Online J. Anim. Feed Res.*, 9(6): 256-259. www.ojafr.ir

Birhan M and Demewez G, Tewodros F, and Tadegenge M.
Online J. Anim. Feed Res., 9(6): 256-259, 2019; pii: S222877011800035-9

Abstract
A cross sectional study was carried out from January, 2018 to June 2018 to determine the abattoir prevalence and economic loss associated with fasciolosis in cattle slaughter at Debretabor municipal abattoir. From the total of 350 examined cattle, 100 (28.6%) were positive for fasciolosis. Highest prevalence was observed in poor body condition cattle 26 (45.6%) followed by medium 54 (26.7%) and good body condition cattle 20 (22%), respectively. There was also significant difference in different age group. The highest 18 (40.9%) prevalence was in young cattle and the lowest 82 (26.8%) found in adult animals. Also the prevalence of bovine fasciolosis was highest in local breed 90 (32.2%) than in cross breed 7 (3.3%) with statistical significant difference ($P < 0.05$). The total annual economic loss was estimated 60746.4 ETB. The study showed that the prevalence and money loss due to fasciolosis in cattle slaughtered at Debretabor municipal abattoir was high. Hence, immediate prevention and control of fasciola is needed.

Keywords: Cattle, Debretabor, Economic significance, Fasciolosis, Prevalence

[Full text-PDF]

Research Paper

Isolation and identification of *Acetobacter* sp. from pineapple (*Ananas comosus* L.) as nata starter.

Khusna A, Prastujati AU, Setiadevi Sh, Hilmi M and Damayanti M.
Online J. Anim. Feed Res., 9(6): 260-264, 2019; pii: S222877011800036-9

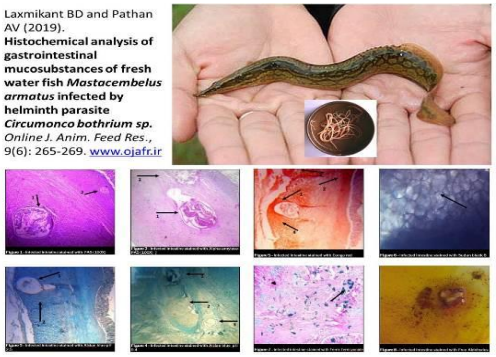
Abstract
The purpose of this study was to isolate and identify the morphological and biochemical properties of *Acetobacter sp*. Isolation is done by growing bacteria taken from pineapple (*Ananas comosus* L.) juice on Tryptic Soy Agar (TSA) media. Identification was carried out by biochemical tests namely catalase, motility, and oxygen use tests. The study was designed per descriptive analysis by evaluating and describing the collected data. The results of the morphological experiments showed that bacterial isolates isolated from pineapple had a milky white color, round shape, small size, smooth surface, flat elevation and gram-negative type. Biochemical tests showed positive reactions in the catalase test because of break-down capability of hydrogen peroxide by the enzyme catalase, while it was negative in the motility test because bacteria form a non-motile free sphere. Bacterial isolates showed a positive reaction in testing the use of oxygen because *Acetobacter sp.* need free oxygen for growth and activity. Isolation of *Acetobacter sp.* pineapple origin has macroscopic characteristics that are milky white color, round shape, smooth surface, and flat elevation. The results of the identification of *Acetobacter sp.* pineapple origin showed a positive reaction to the catalase test, which is a gram-negative bacteria and has a round shape. Future studies are recommended to conduct a polymer chain reaction test (PCR) to identify the strain of *acetobacter sp*.

Keywords: *Acetobacter sp*., Nata, Pineapple, Starter

[Full text-PDF]

Research Paper

Histochemical analysis of gastrointestinal mucosubstances of fresh water fish *Mastacembelus armatus* infected by helminth parasite *Circumonco bothrium* sp.



Laxmikant BD and Pathan AV.
Online J. Anim. Feed Res., 9(6): 265-269, 2019; pii: S222877011800037-9

Abstract
Present study was conducted to investigate the histochemical changes induced by *Circumonco bothrium sp.* in the intestine of freshwater fish *Mastacembelus armatus*. During present investigations the infection of *Circumonco bothrium sp.* in *Mastacembelus armatus* with various histochemical reactions showed localization of localization of carbohydrate, protein, lipid and glycogen. During histochemical study intestine infected by cestodes, the numbers of mucous cells those containing acidic or mixed glycoconjugates were significantly higher than those seen on sections from uninfected fish, which is a protective interaction of the host against parasitic infection. In the current study, a highly significant increase in the number of mucous cells was seen within the infected intestines of *Mastacembelus armatus* when compared to uninfected counterparts.

Keywords: *Circumonco bothrium sp.,* Histochemical, Intestine, *Mastacembelus armatus*

[Full text-PDF]

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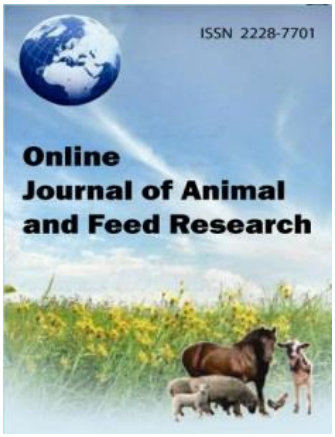

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THE EFFECTS OF SOME MEDICINAL PLANTS WITH INSULIN ON THE INFLAMMATORY AND METABOLIC RESPONSES IN DOGS WITH INDUCED DIABETES MELLITUS

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✎*Supporting Information*

RESEARCH ARTICLE

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ABSTRACT: The goals of this study were to determine biochemical biomarkers and histopathological changes in addition to exploring insulin as mono-therapy as well as insulin plus some medical plants include Garlic, Fenugreek (*Trigonella foenum-graecum* L.) and Black seeds (*Nigella Sativa*) as feed additives in dogs with experimentally induced diabetes mellitus (DM). A total of 15 clinically apparent healthy adult dogs were involved in this study and divided into group 1: included five normal adult dogs served as non-diabetic control group, group 2: Included five adult dogs that were subjected to experimentally diabetes mellitus by intravenous (IV) injection of alloxan and treated by with insulin only and group 3: Included five adult dogs that were subjected to experimentally diabetes mellitus by IV injection of alloxan and treated by with insulin plus once daily supplement with herbal therapy. In conclusions, the therapeutic effects of some medical plants include Garlic, Fenugreek (*Trigonella foenum-graecum* L.) and Black seeds (*Nigella Sativa*) increased the potency and glycemic control of insulin in animals with diabetes mellitus. Therefore, it is recommended adding the previously mentioned herbs as feed additives to improve the potency of insulin to control DM in dogs.

Keywords: Dogs; Diabetes mellitus, Alloxan hydrate; Insulin; Insulin with herbal therapy

INTRODUCTION

Diabetes mellitus (DM) is a common disorder that has been associated with increased morbidity and mortality rate and defined as a metabolic disorder characterized by chronic hyperglycemia and decreased in insulin secretion, insulin action, or both, resulting in impaired carbohydrate, lipid and protein metabolism (Ti Zhang et al., 2006; Ettinger et al., 2017).

Clinical signs of DM appear when blood glucose concentrations of 180–220 mg/dl in dogs and 220–270 mg/dl in cats (Nelson and Reusch, 2014). Diabetic dogs and cats are hyperglycemic and glucosuric with clinical signs of polyuria, polydipsia polyphagia, and weight loss as well as other metabolic alterations including hepatic lipidosis, hepatomegaly, hypercholesterolemia, hypertriglyceridemia, increased catabolism and ketoacidosis (Rucinsky et al., 2010).

The acute phase response is a non-specific inflammatory reaction after any tissue damage and is associated with decreased concentrations of negative acute phase protein such as albumin or transferrin, and/or increased concentrations of positive acute phase protein that include C-reactive protein (CRP), serum amyloid A (SAA), haptoglobin (Hp), α-1-acid glycoprotein, and ceruloplasmin. Most positive acute phase protein, are glycoproteins synthesized by hepatocytes and their secretion is potentiated by pro-inflammatory cytokines (Cero´n et al., 2005). However, the novel role of acute phase protein in DM need further investigation.

Oral hypoglycemic drugs with insulin are used as medications to control diabetes mellitus (Mesa, 2014). Herbal therapy are used by about 60% of the world's population without side effects compared to the traditional plants and play a crucial role in controlling DM, these include *Allium sativum, Eugenia jambolana, Momordica charantia, Ocimum sanctum, Phyllanthus amarus, Pterocarpus marsupium, Tinospora cordifolia, C. indica, Helicteres isora, Stevia rebaudiana, Gymnema sylvestre, Enicostemma littorale Blume* (Patel et al., 2012). Thereby, the role of herbal therapy in DM needs to be elucidated in pet animals.

The aims of this study were to determine the clinical picture, biochemical and histopathological changes on the experimentally-induced diabetic dogs, investigate a recent novel biomarkers in diagnosis of affected animals like determination of acute-phase proteins in addition to investigating insulin as mono-therapy as well as insulin plus some medical plants include Garlic tablets, Fenugreek and Nigella Sativa as feed additives.

Citation: Hassan H, Zaghawa A, Aly M, Kamr A, Nayel M, Mohamed M A-E-G, Abdelazeim A and Hassan B (2019). The effects of some medicinal plants with insulin on the inflammatory and metabolic responses in dogs with induced diabetes mellitus. *Online J. Anim. Feed Res.*, 9(6): 212-224. www.ojafr.ir

MATERIALS AND METHODS

Animals' criteria and ethical approval

A total number of 15 clinically healthy adult dogs were included in this study. Their age ranged from 2.3±0.3 years old and their body weights from 12.5±0.5 kg. They were fed on a diet composed of meat, bones and bread twice daily free access to sufficient tap water. The animals were placed in separated metal cages and kept under the same environmental, nutritional and hygienic condition throughout the period of experiment (4 weeks). All animals were clinically healthy based on physical examination, as well as, some laboratory investigation as: serum biochemistry analysis and urinalysis, to make sure that all dogs under the experimentation are free from both external and internal parasite, plus blood parasite and apparently healthy.

The present study was carried at veterinary hospital of faculty of veterinary medicine Sadat City University. And these animals are divided into three main groups as following: group 1: included five normal adult dogs served as non-diabetic control group and their health condition confirmed by physical and biochemical evaluation at day (D) 1,2 and 3 during induction and treatment. Group 2 included five adult dogs that were subjected to experimentally diabetes mellitus by IV injection of alloxan and treated by insulin (MIXTARD®) S/C in a dose rate of (1.0 IU/kg body weight) once daily for one month and evaluated at D1,2 and 3 during induction and treatment (Hess and Ward, 2000). Group 3: Included five adult dogs that were subjected to experimentally diabetes mellitus by IV injection of alloxan and treated by insulin (MIXTARD®) S/C in a dose rate of (1.0 IU/kg body weight) once daily for one month in addition to Garlic (TOMIX®) one tablet orally plus feed additives purchased from local market (1.5 g/kg/day of Fenugreek seeds powder + 2g/day of Nigella sativa seeds and evaluated at D1 and 2 of induction and treatment. All the procedures in this study was approved by animal Ethics Committee for the Care and Use of Animals in Education and Scientific Research, University of Sadat city (Approval number VUSC-007-1-19).

Experimental induction of diabetes mellitus in dogs

Dogs were fasted for at least 12 hr. before the experimental induction of diabetes, which was produced by single intravenous administration of Alloxan hydrate at dose rate of (35 mg/kg) body weight dissolved in 5cm of normal saline, then 2-3 days later, the dogs showed a fasting blood glucose concentrations of >10 mmol L^{-1} (Christopher et al., 2006), blood glucose concentrations were measured frequently for 7days, by using a (Easy touch) ® G, blood glucose monitoring system. Blood glucose concentrations (over 200mg/dl) were considered diabetic. Within 2-3 days, of clearance of clinical symptoms the diabetic dogs began receiving the insulin subcutaneous injections in a dose rate of rate (1.0 IU)/kg body weight once daily (Hess and Ward, 2000). All dogs had free access to water at all times; the administered diet was composed of a low carbohydrate and high protein and fiber twice daily, at a fixed time before insulin administration to ensure dietary intake before onset of insulin action. All dogs were evaluated by clinical examination and laboratory studies before and after injection of alloxan at (Zero, 2nd, 4 th, 7 th, 9 th, 11 th, 14 th, 18 th days).

Samples

Blood samples were obtained from jugular vein and cephalic vein by sterile needle with large bore. The blood samples were collected without anticoagulant for obtaining clear non-hemolyzed serum by centrifugation of the blood sample at 3000 rpm. For 5 minutes. The clear sera were aspirated carefully by Pasteur pipette and transferred into clean dry labeled Eppendorf tubes and stored at-20 c till examination. Urine samples were collected by catheterization under aseptic condition for urine analysis using urine test stripes according to Brenner et al. (2009). Post mortem liver and pancreatic specimen samples were collected from dead dogs about 0.5 cm thickness at the end of the experiment for histopathology.

Measurements

Serum glucose concentrations (mg/dl) was determined colometrically using of special kits according to the method described by Trinder (1969). Serum insulin concentrations (mg/dl) was determined by radioimmunoassay method using radioimmunoassay kits according to the method described by Wilson and Miles (1977). Serum alanine aminotransferases (ALT) concentrations (U/L) was determined according to the method that described by Young (1990). Serum aspartate aminotransferases (AST) concentrations (U/L) was determined according to the method that described by Reitman and frankle (1957). Serum alkaline phosphatases (ALP) (U/L) was determined according to the method that described by Henderson et al. (2001). Serum cholesterol concentrations (g/dl) was determined by enzymatically method according to Meiattini et al. (1978). Serum triglycerides concentrations (g/dl) was determined by enzymatically method according to Kaplan et al. (1984). Serum HDL and cholesterol and LDL cholesterols (mg/dl) were determined by precipitation method according to Cunical Chemistry (1995). Serum albumin concentrations (g/dl) was determined calorimetrically by using dye-binding technique with Bromcresol green according to Webster (1974). Serum amyloid A concentrations (mg/dl) was determined according to the method described by Supranee jitpean et al. (2014). Serum c-reactive proteins concentrations (µg/ml) was determined according to the method that described by Fransson et al. (2007). Serum haptoglobin concentrations (g/l) was determined according to the method described by Sima Sahinduran et al. (2016).

Citation: Hassan H, Zaghawa A, Aly M, Kamr A, Nayel M, Mohamed M A-E-G, Abdelazeim A and Hassan B (2019). The effects of some medicinal plants with insulin on the inflammatory and metabolic responses in dogs with induced diabetes mellitus. *Online J. Anim. Feed Res.*, 9(6): 212-224. www.ojafr.ir

Histopathological examination

Samples taken from pancreas and liver were collected immediately after death and fixed in neutral buffered formalin 10%. After complete fixation, the specimens were dehydrated, infiltrated and embedded in paraffin. The paraffin blocks were sectioned at 5-7 microns thickness. Tissue were stained with haematoxylin and eosin stain (Thomas and Richter, 1984).

Statistical analysis

Data was normally distributed according to Shapiro-Wilk test and expressed as mean with standard errors. Data was analyzed using T-test and repeated measure ANOVA using SPSS software. Significance was set at $P<0.05$. Area under the curve (ROC) was calculated to determine the sensitivity and specificity of acute phase protein in dogs with induced diabetes mellitus by using SPSS.

RESULTS

Clinical findings between healthy and diabetic groups

The physical examination concerning body temperature revealed non- significant changes between groups, while heart rate revealed a significant decrease in both diabetic groups (2 and 3) compared with those of normal control group 1 (Table 1).

Urine analysis between diabetic and control groups

The diabetic dogs had glycosuria and proteinuria. However, no change in the urine PH was observed compared with normal dogs (Table 1).

Table 1 - Clinical findings and urine analysis in clinically healthy and in experimentally diabetic dogs

Variables / Groups	Control	After induction
Body temperature (C°)	37.5± 0.33[a]	38.3± 0.80[a]
Heart Rate (Beat/Min)	80.5± 2.2[a]	65.4± 3.6[b]
pH	5	5
Glucose	-	+++
Protein	-	+++

Biochemical analysis between diabetic and control groups at 1st week of induction

Table 2 illustrated that serum insulin concentrations were not statistically significant at D1 in all groups, but decreased in concentrations at D2 and D3 in group 2 as well as group 3 compared to control ($P<0.05$). Serum glucose concentrations were not changed significantly at D1 in all groups, but its concentrations reached its peak at D2, D3 of induction in both groups 2 and 3 compared to control ($P<0.05$). Serum enzymatic activities of liver including (ALP, AST and ALT) were not statistically different at first day of induction of diabetes mellitus in groups 2 and 3 than control ($P>0.05$), but significantly elevated at D2 and D3 of induction in groups 2 and 3 compared with control ($P<0.05$). Serum albumin concentrations were not significantly different at D1 and D2 of induction however, its concentration were elevated at D3 of induction compared to control ($P<0.05$). Triglycerides and cholesterol concentrations were not statistically different at first day of induction of diabetes mellitus in group 2 as well as group 3 compared to control group1 ($P>0.05$), but significantly elevated at D2 and D3 of induction in both groups 2 and 3 compared with control group1 ($P<0.05$). Low density lipoprotein (LDL) and High density lipoprotein (HDL) concentrations were not significantly different at D1 of induction in all groups, but serum LDL were elevated and HDL were reduced at D2 and D3 of induction in both groups 2 and 3 compared to control ($P<0.05$). SAA, CRP and haptoglobin concentrations were not statistically different at first day of induction of diabetes mellitus in groups 2 and 3 than control ($P>0.05$). However, SAA, CRP and haptoglobin concentrations were significantly elevated at D2 and D3 of induction in groups 2 and 3 compared with control ($P<0.05$) as shown in Table 2.

Biochemical analysis between diabetic and control groups at 1st week of treatment and 2nd week of induction

Serum insulin concentrations during 1st week of treatment were significantly lower in both treated groups at D1 and D2 than control. However, its concentrations start to increase significantly in group 3 treated with insulin + herbal therapy than group 2 treated with insulin alone ($P<0.05$). Serum glucose concentration during 1st week of treatment still show significant increase in both treated groups at D1, D2 and D3 than control ($P<0.05$, Table 3). Serum concentrations of ALP were significantly higher in both treated groups compared to control ($P<0.05$). Serum ALT and AST were elevated significantly in both treated groups compared to control, but their elevation in group 3 treated with insulin + herbal therapy were significantly lower than group 2 treated with insulin only ($P<0.05$, Table 3). Serum albumin concentrations

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were significantly elevated at D1 and D2 of treatment in both treated groups than control one. However, its concentration were not statistically significant at D3 of treatment in both treated groups compared to control group (P<0.05, Table 3). Serum triglycerides concentrations were elevated significantly in both treated group at D1 and D2 compared to control one, but their elevation in D3 in treated dogs with insulin + herbal therapy were significantly lower than in dogs treated with insulin only (P<0.05). Serum cholesterol concentrations were significantly elevated at D1, D2 and D3 in two treated groups compared to control (P<0.05). Serum LDL were elevated and HDL were reduced significantly at D1, D2 and D3 of treatment in both treated groups compared to control group (P<0.05, Table 3). C-reactive protein and haptoglobin concentrations were significantly elevated at D1, D2 and D3 in two treated groups compared to control (P<0.05). However, SAA were elevated in D1, D2 in two treated groups compared to control except at D3 .which, SAA were not significantly different in the two treated group compared to control group (P>0.05, Table 3).

Table 2 - Biochemical analysis between diabetic and control groups at 1st week of induction

Variables	Weeks	Days	Control	After induction without treatment	
				Insulin	Insulin with herbal therapy
Insulin (µlu/mL)	1st week	D1	10.3±0.6^a	10.2±0.7^a	10.1±0.9^a
		D2	10.2±0.4^a	6.7±0.4^a	6.7±0.1^a
		D3	10.5±0.6^a	4.4±0.3^b	4.5±0.2^b
Glucose (mg/dL)	1st week	D1	95.3±2.6^a	92.2±5.1^a	91.8±5.6^a
		D2	89±5.5^a	150.4±8.01^b	159.4±5.6^b
		D3	90.8±5.6^a	203±7.5^b	206.2±7.3^b
ALP (u/L)	1st week	D1	155.8±0.7^a	153.8±0.8^a	156.4±0.4^a
		D2	161.6±0.5^a	193.4±0.6^b	198.8±0.8^b
		D3	160.4±0.5^a	248.6±0.7^b	253.6±0.12^b
AST (u/L)	1st week	D1	33.8±2.1^a	35.2±3.2^a	32.2±2.3^a
		D2	32±3.1^a	54.8±2.3^b	57.2±3.33^b
		D3	36.4±2.7^a	72.2±3.8^b	72.8±5.9^b
ALT (u/L)	1st week	D1	33.4±1.3^a	32.2±2.1^a	31.6±2.2^a
		D2	31±1.6^a	54.8±2.8^b	52.6±3.1^b
		D3	31.2±1.5^a	79.8±3.8^b	74.6±4.3^b
Albumin (mg/dL)	1st week	D1	4.3±0.2^a	4.3±0.3^a	4.2±0.3^a
		D2	4.1±0.2a	4.1±0.4^a	4.02±0.1^a
		D3	4.3±0.2^a	5.8±0.2^b	6.14±0.1^b
Triglycerides (mg/dL)	1st week	D1	125±1.6^a	113.6±2.1^a	111.4±3.6^a
		D2	117.4±4.5^a	148.6±7.7^b	141.4±3.6^b
		D3	117.2±4.5^a	189.4±8.7^b	184.4±3.6^b
Cholesterol (mg/dL)	1st week	D1	157.4±6.4^a	148.1±4.8^a	150.4±4.8^a
		D2	155.6±3.7^a	169.4±4.5^b	174.2±5.1^b
		D3	151.2±6.01^a	225.2±8.3^b	217.8±5.8^b
LDL (mg/dL)	1st week	D1	76.6. ±5.6^a	74.2±3.3^a	74.4±4.4^a
		D2	76.7±2.9^a	108.2±3.5^b	115.8±2.9^b
		D3	77.8±2.7^a	151.8±2.2^b	159±3.63^b
HDL (mg/dL)	1st week	D1	58.6±1.4^a	57.4±6.1^a	58.6±5.1^a
		D2	62.2±5.5^a	50.4±6.03^b	50.8±4.7^b
		D3	59.6±5.7^a	31.6±3.8^b	32.1±3.7^b
SAA (µg/mL)	1st week	D1	4.7±0.1^a	5.3±0.3^a	5.1±0.2^a
		D2	5.1±0.2^a	7.4±0.1^b	7.5±0.2^b
		D3	5.16±0.3^a	8.3±0.3^b	8.4±0.2^b
CRP (mg/L)	1st week	D1	3.9±0.2^a	4.1±0.4^a	4.3±0.3^a
		D2	4.2±0.4^a	8.3±0.6^b	7.5±0.1^b
		D3	4.2±0.4^a	10.8±0.4^b	11.4±0.4^b
Haptoglobin (g/L)	1st week	D1	0.96±0.1^a	1.08±0.2^a	0.98±0.1^a
		D2	1.08±0.2^a	2.9±0.4^b	2.9±0.6^b
		D3	1.12±0.1^a	4.22±0.4^b	5.04±0.5^b

ALP-alkaline phosphatase; AST-aspartate aminotransferase; ALT- alanine aminotransferase; LDL-low density lipoprotein; HDL- high density lipoprotein; SAA-serum amyloid A; CRP-c-reactive protein. Data are presented as means ± standard error (S.E).Mean value with different superscript letters in the same row are significantly different at P<0.05.

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Table 3 - Biochemical analysis between diabetic and control groups at 1st week of treatment and 2nd week of induction

Variables	Weeks	Days	Control	After induction without treatment	
				Insulin	Insulin with herbal therapy
Insulin (µlu/mL)	2nd week	D1	10.04±0.3^{a}	3.98±0.2^{b}	3.94±0.1^{b}
		D2	10.18±0.4^{a}	6.16±0.3^{b}	6.98±0.4^{b}
		D3	10.50±0.4^{a}	6.72±0.1^{b}	8.44±0.2^{a,b}
Glucose (mg/dL)	2nd week	D1	93.0±1.2^{a}	275.2±3.2^{b}	275.6±4.8^{b}
		D2	91.80±1.5^{a}	221.80±4.6^{b}	233.60±5.3^{b}
		D3	89.0±1.8^{a}	207.40±3.9^{b}	209.0±4.2^{b}
ALP (u/L)	2nd week	D1	161.6±3.4^{a}	271.6±5.6^{b}	281.0±6.2^{b}
		D2	153.80±3.2^{a}	253.60±4.5^{b}	257.0±6.1^{b}
		D3	159.0±3.2^{a}	234.20±7.4^{b}	235.60±5.8^{b}
AST (u/L)	2nd week	D1	32.0±1.9^{a}	93.60±2.1^{b}	93.80±3.2^{b}
		D2	35.20±2.1^{a}	81.80±3.4^{b}	74.20±2.5^{a,b}
		D3	34.40±2.3^{a}	69.80±3.4^{b}	54.60±3.6^{a,b}
ALT (u/L)	2nd week	D1	31.80±1.3^{a}	114.40±2.5^{b}	105.80±2.8^{b}
		D2	32.20±1.8^{a}	100.4±3.2^{b}	88.60±3.4^{a,b}
		D3	31.0±1.6^{a}	88.80±2.3^{b}	66.0±2.3^{a,b}
Albumin (mg/dL)	2nd week	D1	4.26±0.1^{a}	5.94±0.3^{b}	6.24±0.1^{b}
		D2	4.26±0.2^{a}	5.56±0.2^{b}	5.84±0.3^{b}
		D3	4.66±0.1^{a}	5.20±0.2^{a}	5.18±0.4^{a}
Triglycerides (mg/dL)	2nd week	D1	111.80±1.8^{a}	210.4±2.5^{b}	210.8±4.1^{b}
		D2	113.60±3.2^{a}	191.0±4.5^{b}	180.0±5.3^{b}
		D3	117.40±2.4^{a}	176.20±6.01^{b}	157.20±5.8^{a,b}
Cholesterol (mg/dL)	2nd week	D1	155.0±3.2^{a}	243.0±4.6^{b}	237.40±7.01^{b}
		D2	148.0±4.9^{a}	220.40±6.2^{b}	214.20±4.3^{b}
		D3	153.60±5.01^{a}	209.20±3.5^{b}	190.20±5.1^{b}
LDL (mg/dL)	2nd week	D1	72.20±3.1^{a}	186.20±3.4^{b}	183.60±5.4^{b}
		D2	74.20±4.3a^{a}	167.40±3.7^{b}	166.40±3.8^{b}
		D3	78.6±2.8^{a}	154.80±2.5^{b}	149.40±4.2^{b}
HDL (mg/dL)	2nd week	D1	62.20±1.4a	21.80±5.2^{b}	23.60±5.6^{b}
		D2	58.60±3.2^{a}	27.0±4.1^{b}	30.40±5.3^{b}
		D3	62.40±4.2^{a}	32.20±3.7^{b}	40.0±4.6^{b}
SAA (µg/mL)	2nd week	D1	5.3±0.2^{a}	9.34±0.6^{b}	10.42±0.8^{b}
		D2	5.08±0.4^{a}	7.22±0.6b	8.24±0.3^{b}
		D3	5.28±0.1^{a}	6.30±0.3^{a}	6.36±0.2^{a}
CRP (mg/L)	2nd week	D1	3.98±0.1^{a}	17.60±1.2^{b}	16.80±1.02^{b}
		D2	4.34±0.2^{a}	15.0±1.01^{b}	14.20±2.1^{b}
		D3	4.20±0.1^{a}	12.80±0.8^{b}	11.40±0.7^{b}
Haptoglobin (g/L)	2nd week	D1	1.10±0.2^{a}	5.32±0.1^{b}	6.84±0.3^{b}
		D2	0.98±0.1^{a}	4.68±0.1^{b}	6.28±0.3^{a,b}
		D3	1.22±0.1^{a}	4.16±0.2^{b}	4.66±0.1^{b}

ALP-alkaline phosphatase; AST-aspartate aminotransferase; ALT- alanine aminotransferase; LDL-low density lipoprotein; HDL- high density lipoprotein; SAA-serum amyloid A; CRP-c-reactive protein. Data are presented as means ± standard error (S.E).Mean value with different superscript letters in the same row are significantly different at P<0.05.

Biochemical analysis between diabetic and control groups at 2nd week of treatment and 3rd week of induction

Serum insulin concentrations were increased significantly in group 2 as well as group 3 compared with those of control values in group 1, whereas its concentrations in (insulin + herbal therapy, group 3) treated animals were significantly higher and reach its normal peak than those of (group 2) treated with insulin only (P<0.05; Table 4). Serum glucose concentrations in (group 3) were significantly decreased in D1 and D2 of 2nd week of treatment compared to (group 2) or control (group 1) (P<0.05; Table 4). Serum enzymatic activities of liver including (ALP, AST and ALT) and serum albumin during 2nd week of treatment. ALP, AST and ALT concentrations were significantly increased in D1 in diabetic groups in comparing with control group at 1st week of induction. Also the previous parameters were significantly lower and return to their normal concentrations in D2 in treated group with insulin + herbal therapy than treated one with insulin only (P<0.05; Table 4). Serum albumin concentrations was significantly higher in both diabetic groups at D1 compared to control group, but were not significantly different at D2 compared to control group as it back to its normal concentrations in both treated group (P>0.05; Table 4). Triglycerides and cholesterol were significantly lower in dogs treated with (insulin + herbal therapy) in D1 and D2 compared to control group and the one treated with insulin only. Serum LDL concentrations were elevated in both diabetic groups significantly than those of the control normal group. But, the increase concentrations in group treated with herbal therapy was significantly lower than those diabetic group treated with insulin only. Serum HDL concentrations were lower in both diabetic groups than control group. But, its concentration in dogs treated with (insulin + herbal therapy) at D2 was significantly higher than those treated with insulin only (P<0.05). SAA concentrations were not significantly different between groups of dogs at D1 and D2 as it back to normal values

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(P>0.05). However, CRP and HP concentrations were elevated significantly in two treated groups of dogs at D1 compared to control group. But the elevation in HP and CRP concentrations in treated group with insulin+ herbal therapy were significantly lower than treated group with insulin only at D2 (P<0.05; Table 4). Insulin concentrations were increased, while glucose concentrations were decreased in insulin with herbal therapy treated group compared with insulin treated group (P<0.05; Figure 1). Other biochemical parameters and acute phase proteins were closely return to normal level significantly in insulin with herbal therapy compared with insulin treated group (P<0.05; Figures 2 and 3).

Table 4 - Biochemical analysis between diabetic and control groups at 2nd week of treatment and 3rd week of induction

Variables	Weeks	Days	Control	After induction without treatment	
				Insulin	Insulin with herbal therapy
Insulin (µu/mL)	3rd week	D1	10.20±0.70^{a}	7.40±0.2^{b}	10.24±0.28^{b}
		D2	10.5±1.4 ^{a}	8.4±0.6^{b}	11.4±1.2 ^{a,b}
Glucose (mg/dL)	3rd week	D1	92.20±5.6^{a}	188.60±5.8^{b}	170.80±2.0^{a,b}
		D2	96±2.4^{a}	170±5.8^{b}	145±3.7^{a,b}
ALP (u/L)	3rd week	D1	156.20±4.6^{a}	224.40±7.4^{b}	208.0± 7.7^{b}
		D2	163±5.4^{a}	205±4.2^{b}	176±7.6^{a}
AST (u/L)	3rd week	D1	32.20±3.2^{a}	60.80±2.7^{b}	39.20±2.08^{b}
		D2	33±1.3 ^{a}	54±2.5^{b}	36±2.8 ^{a,b}
ALT (u/L)	3rd week	D1	31.60±2.24^{a}	75.80±3.4^{b}	48.0±2.5^{a,b}
		D2	32±1.6 ^{a}	67±0.2^{b}	39±0.4^{a,b}
Albumin (mg/dL)	3rd week	D1	4.30±0.35^{a}	5.24±0.26^{b}	4.80±0.14^{b}
		D2	4.3±0.3 ^{a}	4.3±0.4 ^{a}	4.9±0.8 ^{a}
Triglycerides (mg/dL)	3rd week	D1	111.40 ±4.2^{a}	163.60 ±9.5^{b}	137.0± 4.4^{a,b}
		D2	113±5.2^{a}	144±4.3^{b}	117±6.4^{a}
Cholesterol (mg/dL)	3rd week	D1	150.40 ±4.8^{a}	197.4±4.2^{b}	168.60±3.6^{a,b}
		D2	149±5.1 ^{a}	187±6.2 ^{b}	150±4.3 ^{a}
LDL (mg/dL)	3rd week	D1	74.20±3.3^{a}	143.80±2.83^{b}	123.40±3.31^{a,b}
		D2	73±1.2 ^{a}	131±2.6 ^{b}	96±3.05^{a,b}
HDL (mg/dL)	3rd week	D1	57.40±6.1^{a}	45.7±3.5^{b}	48.80± 1.7^{b}
		D2	53±1.3 ^{a}	31±1.2^{a,b}	48±2.6 ^{b}
SAA (µg/mL)	3rd week	D1	5.36 ±0.32^{a}	5.52±0.24^{a}	5.66±0.12^{a}
		D2	5.2±0.1^{a}	5.5±0.3^{a}	5.3±0.7^{a}
CRP (mg/L)	3rd week	D1	4.10±0. 47^{a}	11.40± 1.2^{b}	9.0±1.4^{b}
		D2	4.2±0.3 ^{a}	9.8±1.2 ^{b}	6.02±0.8 ^{a,b}
Haptoglobin (g/L)	3rd week	D1	1.08±0.24^{a}	3.14±0.21^{b}	2.78±0.08^{b}
		D2	0.8±0.5 ^{a}	2.5±0.6^{b}	1.6± 0.7^{a,b}

ALP-alkaline phosphatase; AST-aspartate aminotransferase; ALT- alanine aminotransferase; LDL-low density lipoprotein; HDL- high density lipoprotein; SAA-serum amyloid A; CRP-c-reactive protein. Data are presented as means ± standard error (S.E).Mean value with different superscript letters in the same row are significantly different at P<0.05.

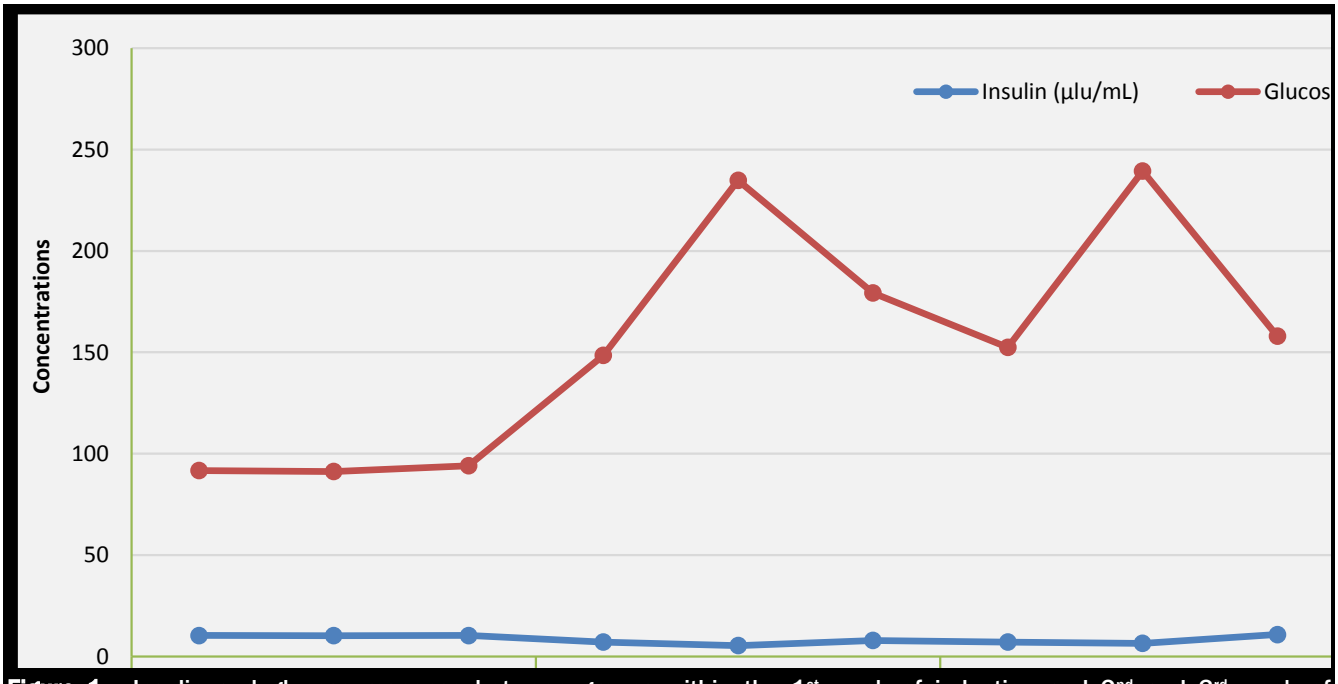

Figure 1 - Insulin and glucose response between groups within the 1st week of induction and 2nd and 3rd week of treatment. Values expressed as mean with standard error.

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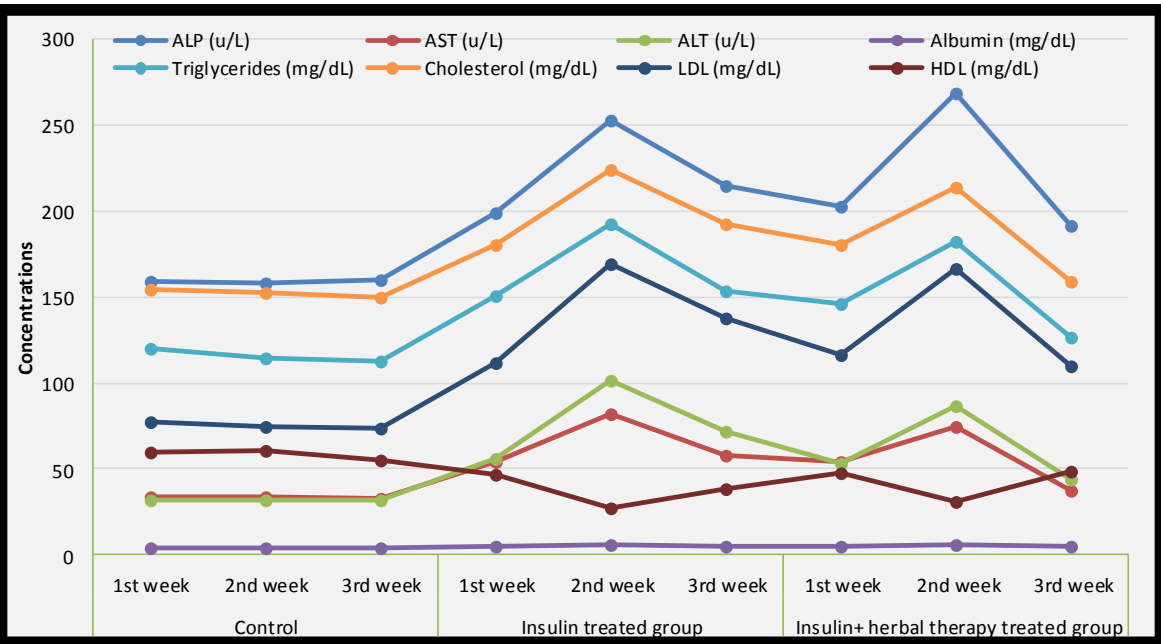

Figure 2 - Biochemical changes between groups within the 1st week of induction and 2nd and 3rd week of treatment. Values expressed as mean with standard error.

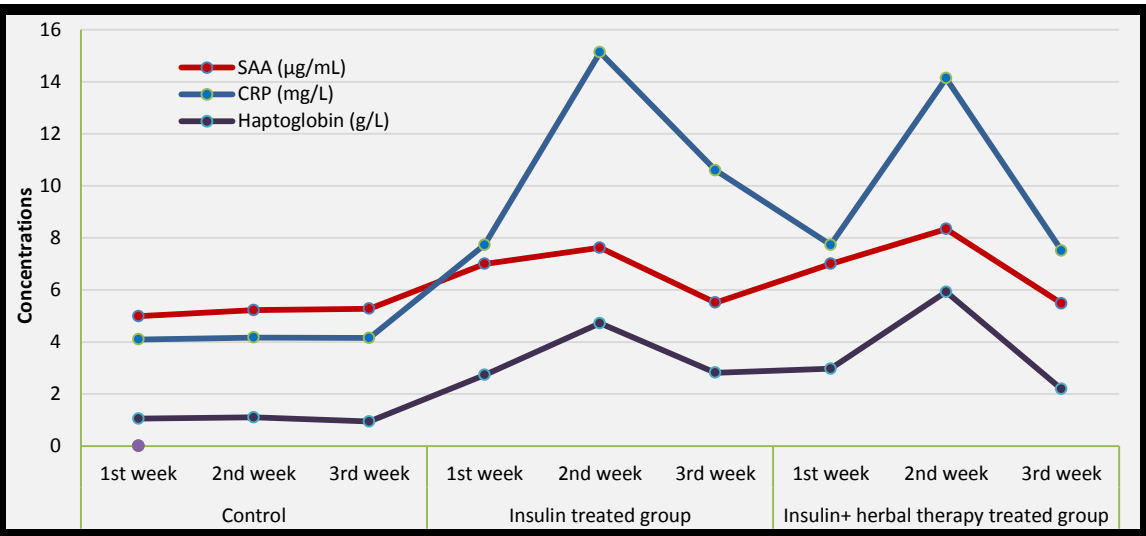

Figure 3 - Acute phase protein pattern between groups within the 1st week of induction and 2nd and 3rd week of treatment. Values expressed as mean with standard error.

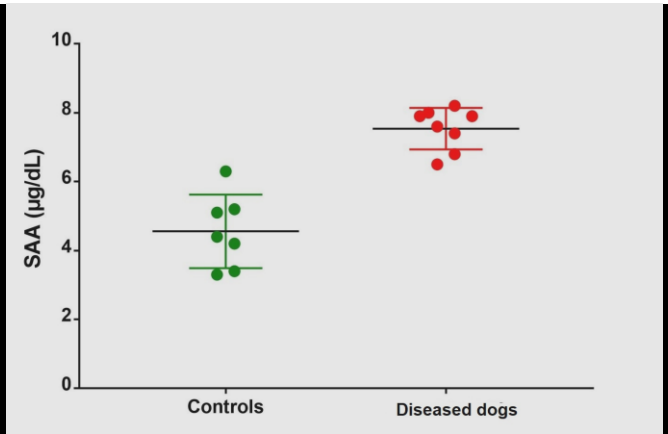

Figure 4 - SAA area under the curve =0.955 with sensitivity =90% and specificity= 85% in dogs with serum amyloid A concentrations = 6.6 µg/mL

Specificity and Sensitivity of acute phase proteins in dogs with experimentally induced diabetes mellitus

Serum amyloid A, c-reactive protein and haptoglobin concentrations were highly sensitive and specific in the diagnosis of dogs with induced diabetes mellitus with area under the curve (AUC) close to 1 with P<0.05 as shown in figure 4.

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Histopathological finding

Pancreatic tissue of dog from group 2 showed different pathological changes like sever congestion of blood vessels, sever coagulative necrosis of pancreatic acini either focal or diffuse, mononuclear cells infiltration between the pancreatic acini, some cells showed programed cell death (apoptosis). Finally, there was sever proliferation of connective tissue (Figure 5 A,B,C&D). Pancreatic tissue of dog from group 3 showed less sever pathological changes represented as congested blood vessels mononuclear cells infiltration (Figure 5 E&F). Hepatic tissue of dog from group 2 showed different pathological changes represented as dilated and congested central vein and hepatic sinusoids, proliferation of Kupffer cells, focal and diffuse mononuclear cells infiltration in the portal areas and hepatic parenchyma. There was also sever swelling of hepatocytes and focal areas of necrosis and mononuclear cells infiltration. The portal areas showed mononuclear cells infiltration and congested blood vessels with proliferation of connective tissue (Figure 6 A,B,C&D). Hepatic tissue of dog from group 3 showed less sever changes represented as congested blood vessels and hepatic sinusoids, the hepatic tissue showed fatty change appeared as clear vacuoles in the hepatocytes, also there was single cell necrosis in the hepatic parenchyma (Figure 6 E&F).

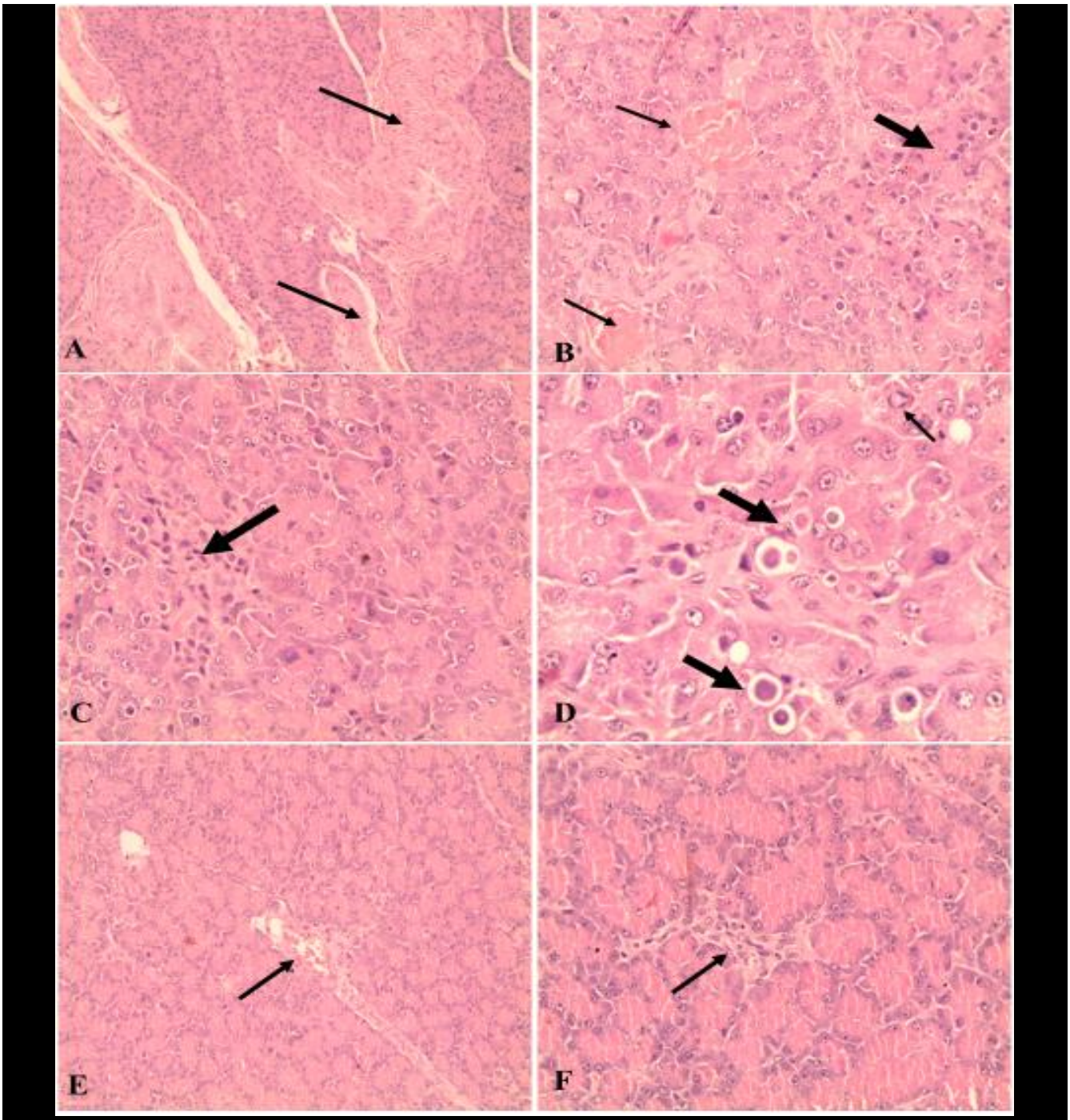

Figure 5 - **A**. Pancreas of dog from (group 2) showed sever fibrosis (arrow) with coagulative necrosis of pancreatic tissue (H&E x 10); **B**. Pancreas of dog from (group 2) showed sever congestion of blood vessels (thin arrow) and sever coagulative necrosis of pancreatic acini (thick arrow) (H&E x 20); **C**. Pancreas of dog from (group 2) showed focal area of necrosis with mononuclear cells infiltration (arrow) (H&E x 20); **D**. Pancreas of dog from (group 2) showed apoptotic cells (thin arrow) and necrotic cells (thick arrow) (H&E x 40); **E**. Pancreas of dog from (group 3) showed congested blood vessels (arrow) (H&E x 10); **F**. Pancreas of dog from (group 3) showed mononuclear cells infiltration (arrow) (H&E x 20).

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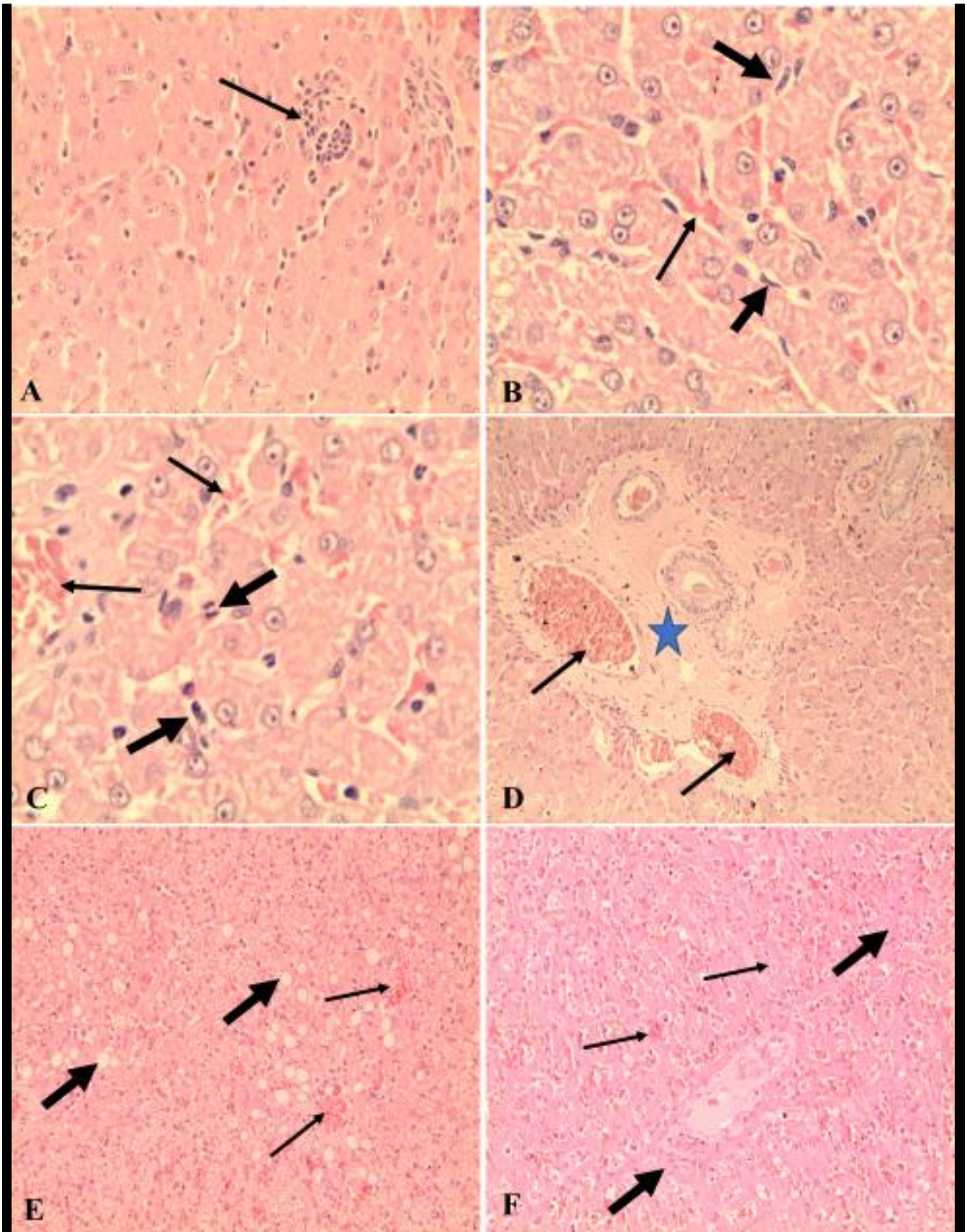

Figure 6 - A. liver of dog from (group 2) showed focal area of necrosis and mononuclear cells infiltration (arrow) (H&E x 20); **B**. liver of dog from (group 2) showed dilated and congested hepatic sinusoids (thin arrow) and proliferation of Kuepfer cells (thick arrow) (H&E x 40); **C**. liver of dog from (group 2) showed congested blood vessels and hepatic sinusoids (thin arrow), diffuse mononuclear cells infiltration (thick arrow) and sever swelling of hepatocytes (H&E x 40); **D**. liver of dog from (group 2) showed proliferation of connective tissue in the portal areas (star) with mononuclear cells infiltration and congested blood vessels (arrow) (H&E x 10); **E**. liver of dog from (group 3) showed congested blood vessels and hepatic sinusoids (thin arrow) and the hepatocytes showed fatty change (thick arrow) (H&E x 10); **F**. liver of dog from (group 3) showed severely congested blood vessels and hepatic sinusoids (thin arrow) and coagulative necrosis in hepatic parenchyma (thick arrow) (H&E x 10).

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DISCUSSION

In the present study administration of single dose of alloxan (35 mg/kg $^{-1}$) induced diabetes mellitus in dogs. One week after alloxan injection, the dogs showed polyuria, polydipsia and polyphagia. Some of these animals were suffered from vomition, dehydration and variable degrees of lethargy, partial anorexia and mild to moderate weight loss. These clinical signs were similar to those reported by Khoshvaghti and Heidi (2012) and Ismail et al. (2015).

There were a significant decrease in heart rate, and non-significant change in the body temperature, before and after the experimental induction of diabetes mellitus, our observations are in agreement with a previous study by Howarth et al. (2011) who revealed that heart rate declined rapidly after administration of alloxan. Serum analysis of alloxan-induced diabetic dogs showed significant increase in fasting blood glucose and decreased insulin concentrations compared to their concentrations before induction of diabetes. This result indicates that alloxan successfully induced DM in dogs as documented by Valilou and Lotfi (2012), Ismail et al. (2015) and Khanam and Dewan (2008).

The liver related enzymes ALT, AST, and ALP concentrations were significantly increased after diabetes induction at D2 and D3 of induction compared with control healthy group. These results were coincided with Valilou and Lotfi (2012), Bhargavi et al. (2015) and Shruthi et al. (2017) who found increase in the concentrations of liver enzymes due to leakage of this enzyme from liver cytosol to blood circulation due to hepatic damage also another suggested reason for increase in AST activity following diabetes is greater need for gluconeogenic substrate due to diabetic condition stated by Tanaka et al. (1988), although elevation of both enzymes ALT, AST concentrations may also reflect the damage of hepatic cells via injected alloxan stated by Joo-min Kim et al. (2006). On the other hand, Ismail et al. (2015) showed a progressive increase in serum concentration of ALP in DM dogs while serum concentrations of other liver enzymes such as AST and ALT were not changed significantly.

Serum concentrations of ALP, ALT and AST were significantly higher in insulin treated animal group compared to control ($P<0.05$) after 2nd week of treatment but there concentrations decreased significantly when compared to the 1st week of induction. ALP, AST and ALT concentrations were significantly lower and return to their normal concentrations in D2 in treated group with insulin + herbal therapy group 3 than group 2 treated with insulin only ($P<0.05$). These results indicated that the administration of insulin with herbal therapy to diabetic dogs was found to be effective in improving liver function tests than insulin treatment alone. These results were coincided with Masjedi et al. (2013), Asdaq (2015) and Johnson et al. (2015) who found a significant reduction in the activities of liver enzymes with garlic in diabetic rats. The protective effects of garlic extract may be associated with the inherent-antioxidant properties as shown by Rahman (2003).

The lipid profile parameters including, cholesterol, triglycerides and LDL were significantly increased after diabetes induction at D2 and D3 of induction compared with control healthy group. Furthermore, we documented that Triglycerides, Serum LDL concentrations and cholesterol were significantly reduced in (group 3) treated with (insulin + herbal therapy) in D1 and D2 compared to control group and (group 2) treated with insulin only. The administration of insulin with herbal therapy to the diabetic dogs was found to be more effective in lowering total cholesterol value, serum LDL concentrations and serum triglycerides value than in insulin administration only. Serum HDL concentrations were lower in both diabetic groups than control group. But, its concentration in (group 3) treated with (insulin + herbal therapy) at D2 was significantly higher than those of (group 2) treated with insulin only. These results were coincided with Masjedi et al. (2013) and Asdaq (2015) who reported significant reduction in triglycerides and total cholesterol with garlic administration in diabetic rats.

The concentrations of acute-phase proteins (APPs) include haptoglobin, C-reactive protein and serum amyloid A started to be elevated significantly at D2 and D3 of induction in (group 2) and (group 3) compared with control group. These results were in agreement with Eckersall, (1995) and Riaz (2015). CRP and HP concentrations were elevated significantly in two treated groups of dogs at D1 compared to control group, but their elevation in treated group with insulin+ herbal therapy (group 3) were significantly lower than treated group with insulin only (group 2) at D2. These results were agreement with Kumar et al. (2013).

Insulin-treated animals showed a significant decrease in blood glucose and increased insulin concentrations in alloxan-induced diabetic dogs in 2nd week treatment. The results were coincided with Mosseri et al. (2000) and Moller (2001). Interestingly serum insulin concentrations were increased significantly in group 2 as well as group 3 compared with those of control values in group 1, whereas its concentrations in (insulin + herbal therapy) (group 3) treated animals were significantly higher and reach its normal peak than those of (group 2) treated with insulin only ($P<0.05$). This result was in agreement with Londhe et al. (2011) who found that Allicin is one of active chemical constituent of garlic that raise the serum insulin concentrations. Additionally, serum glucose concentrations in (group 3) were significantly decreased in D1 and D2 of 2nd week of treatment compared to (group 2) or control (group 1). The administration of insulin with herbal therapy to diabetic animals was found to be more effective in lowering blood glucose concentrations than insulin administration alone. This result agreement with Kumar et al., (2013), Masjedi et al. (2013) and johnson et al. (2015) who found that garlic showing a significant decrease in serum glucose concentrations alone or in combination with either anti-diabetic drugs or other herbal therapy.

The histopathological examination of the pancreas of alloxan-induced diabetic dogs treated with insulin plus herbal therapy (group 3) revealed less sever pathological changes compared with (group 2) treated with insulin only, represented as congested blood vessels mononuclear cells infiltration.

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The histopathological examination of the pancreas of insulin treated group revealed sever congestion of blood vessels, sever coagulative necrosis of pancreatic acini either focal or diffuse, mononuclear cells infiltration between the pancreatic acini, some cells showed programed cell death (apoptosis). Finally, there was severing proliferation of connective tissue. These histopathological finding were coincided with Joo-Min Kim et al. (2006), Khanam and Dewan (2008) and Ismail et al. (2015). Moreover, The histopathological examination of the liver of insulin treated group revealed different pathological changes represented as dilated and congested central vein and hepatic sinusoids, proliferation of Kupffer cells, focal and diffuse mononuclear cells infiltration in the portal areas and hepatic parenchyma. There was also sever swelling of hepatocytes and focal areas of necrosis and mononuclear cells infiltration. The portal areas showed mononuclear cells infiltration and congested blood vessels with proliferation of connective tissue These finding were consistent with these reported by Joo-Min Kim et al. (2006), Khanam and Dewan (2008) and Ismail et al. (2015).

Of interest, The histopathological examination of the liver of alloxan-induced diabetic dogs treated with insulin plus herbal therapy (group 3) revealed less sever pathological changes compared with (group 2) treated with insulin only, represented as congested blood vessels and hepatic sinusoids, the hepatic tissue showed fatty change appeared as clear vacuoles in the hepatocytes, also there was single cell necrosis in the hepatic parenchyma. These results were agreement with Masjedi et al. (2013) who conclude that the inhibition of morphology and histomorphometrical changes in the pancreas and the better improvement in liver tissue were dramatically decreased due to garlic juice administration are considered to be the direct evidence that garlic juice improves diabetes. Also there was an improvement in the microscopic picture of examined pancreas, liver and kidney in diabetic rates treated with *Trigonella foenum* by Abou El-Soud et al. (2007) who mentioned that the hepatic lobules appeared more or less like control and Kanter et al. (2004) who founded NS treatment has been provide a protective effect by decreasing lipid peroxidation and serum NO, and increasing antioxidant enzyme activity. Islet cell degeneration and weak insulin immunohistochemical staining was observed in rats with STZ-induced diabetes. Increased intensity of staining for insulin, and preservation of beta-cell numbers were apparent in the NS-treated diabetic rats. These findings suggest that NS treatment exerts a therapeutic protective effect in diabetes by decreasing oxidative stress and preserving pancreatic beta-cell integrity. Consequently, NS may be clinically useful for protecting beta-cells against oxidative stress. This study has a limitation lacking of day 3 at 2nd week of treatment and 3rd week of induction.

CONCLUSION

Acute-phase proteins (APPs) can be used as diagnostic biomarkers in DM and early acute pancreatitis. Moreover, the therapeutic effects of the medical plants like (Garlic, Fenugreek and Black seeds) were increased the potency and glycemic control of insulin in animals with diabetes mellitus. Therefore these plants are recommended to be used for treatment of DM in animals because of their hypoglycemic effect. However, this study has a limitation lacking of group of animals treated with herbal therapy, this study could give a future support for using of herbal therapy as potential medicine beside insulin to control diabetes mellitus in dogs.

DECLARATIONS

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Author's contribution

All authors were contributed equally in this study

Competing interests

The authors declare that they have no competing interests.

Conflict of interest

The authors declared that no conflict of interest

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PREVALENCE AND ASSOCIATED RISK FACTORS OF CYSTICECHINOCOCCOSIS IN PIGS SLAUGHTERED AT ADDIS ABABA ABATTOIR ENTERPRISE

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Supporting Information

RESEARCH ARTICLE

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ABSTRACT: A cross sectional study was conducted from January 2018 to April 2018 to determine the prevalence and associated risk factors of cystic echinococcosis in pig slaughtered at Addis Ababa abattoir enterprise, central part of Ethiopia. A total of 251 pigs were randomly sampled and routine meat inspection procedure was employed to detect the presence of hydatid cyst in the visceral organs (lung, liver, spleen and kidney), where 25 (9.96%) pigs were positive. Analysis of risk factors for occurrence of the disease revealed that there was statistically significant variation (P<0.05) in swine with different body condition scores and age groups. However, significant variation was not observed (P>0.05) across different sex and origin. Prevalence of distribution of hydatid cyst in different organs from total examined swine were 3.59%, 3.19%, 1.99%, 0.80%, 0.40%, for lungs and livers, livers, lungs, spleens and kidney respectively. In this study, the liver was found to be the most predominantly affected organ (6.77%) followed by the lungs (5.58%), spleen (0.80%) and the least affected organ was kidney (0.40%). As regards size of the cyst from total infected organs, organ with small sized cysts had the highest percentage (67.6%), followed by medium sized cysts (20.6%) and large sized cysts (11.8%). Livers (44.1%) were predominant organ infected with small cyst size while spleen (5.9%) and kidney (2.9%) have only small cyst size. Lungs (14.7%) and liver (5.9%) were infected with medium cyst size while only lungs (11.8%) have large cyst size. From the total of 34 (100%) affected organs, only 4 (11.8%) lungs have more than or equal to three cyst numbers while remaining 30 (88.2 %) affected organs were with less than three cyst numbers.

Keywords: Cyst number, Cyst size, Echinococcosis, Echinococcus granulosus, Hydatid cysts, Prevalence, Risk factors.

INTRODUCTION

Pigs, also called as hogs or swine, are ungulates which have been domesticated as a source of food, leather and similar products since ancient times. More in recent times, they have been involved in biochemical research and treatment (Pam et al., 2013). Swine production forms a fundamental part of farmer's economy in many parts of the world. Many countries practice different kinds of production approaches. Swine production is increasing from time to time in many parts of tropical countries. An increased demand on international market, due to increased number of pork consumer and the profit obtained from the sector make the production to increase rapidly (Serres, 2001). However, pig production in developing countries is contributing little benefits due to many production constraints including; under developed infrastructure, poor genetic performance of local breeds, insufficient nutrition, poor management and husbandry practices, shortage of trained man power, cultural and religious taboo on marketing and consumption of pork and wide spread diseases (FAO, 2000).

Pig production in Ethiopia is to be in its newborn stage until current day. The pig population in the country is estimated to be 29,000 heads representing 0.1% of African pig population (FAO, 2005). In many rural parts of Ethiopia, pig production was characterized by extensive production system whereby animals are allowed to forage at backyard and municipal debris discarding sites (Abdu and Gashaw, 2010). On the other hand, extensive husbandry system coupled with poor environmental hygiene and voracious feeding behavior of pigs have been indicated as a major risk factor for infection of pigs with helminthes and gastrointestinal parasites where pigs may act as potential reservoir hosts of human gastro-intestinal parasites such as ascaris (Irvin, 2003).

Cystic echinococcosis (CE) is one of the most important helminthes zoonosis and rests as a major problem worldwide. It is caused by the larval stages of the tapeworm *Echinococcus granulose*, which is found in the small intestines of dogs and other carnivores. It is also is known to be one of the most important parasitic infections in livestock worldwide (Craig et al., 2007). In Ethiopia, hydatidosis (Cystic echinococcosis in pig) is one of the major parasitic zoonotic diseases particularly where pigs, shoat, cattle and camel are still slaughtered traditionally and offal's are effortlessly accessible to scavenging dog and other wild carnivores. Factors like absence of proper meat inspection procedure, poor supervision of food animals, traditional practices of back yard farming system, lack of attentiveness about food borne

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disease and presence of large stray dog population contribute significantly to the prevalence of the disease in Ethiopia (Yetinayet, 2010).

The parasite has an indirect life cycle utilizing dogs and other canids as definitive hosts and many herbivorous and omnivorous species, including wildlife and domesticated livestock as intermediate hosts. The tapeworm spends most of its adult life in the intestine of its definitive host, namely canids in particular dogs. The tapeworm eggs become voided in the canids' faeces and as a result of ingesting the eggs, infection passes to the intermediate host, commonly herbivores while grazing. However, humans can become accidentally infected and hydatid cysts may develop throughout the body (Ahmadi and Meshkehkar, 2011). As it is indicated in numerous medical literatures, hydatidosis is also a well-recognized zoonosis (Ali et al., 2008). In each year due to hydatidosis, a significant amount of economic loss results from death of animals, inferior weight gain, condemnation of edible organs and carcass at slaughter houses. This production loss to the livestock industry is expected to be more than 900 million USD annually (Andualem, 2007).

Factors governing the prevalence of hydatidosis in a given district may be associated with prevailing specific social, cultural, environmental and epidemiological conditions. It has a considerable socio-economic effect in countries where livestock industry is an important segment of agricultural sector and when livestock production is based mainly on extensive grazing system (Berhe, 2009). Similarly its significance is higher in developing countries particularly in rural communities where there is a close contact between dogs (definitive host) and various domestic animals which are intermediate host (Eckert and Deplazes, 2004; Eckert et al., 2001). *Echinococcus granulosus*, a causative agent of hydatidosis, is wide spread in Ethiopia with great economic and public health significance (Sisay et al., 2008; Nigatu et al., 2009). In spite of the large efforts that have been put into the research and control of echinococcosis, it still remains a disease of worldwide significance. In some areas of the world, Cystic echinococcosis caused by *E. granulosus* is a re-emerging disease in places where it was previously at low levels (Torgerson and Budke, 2003).

The general objective of the study was to know the current prevalence and associated risk factors of cystic echinococcosis in pigs slaughtered at Addis Ababa abattoir enterprise. The study aimed to determine the current prevalence status of cystic echinococcosis in pig and also to identify responsible risk factors for the prevalence of hydatidosis.

MATERIALS AND METHODS

Study area

The study was conducted at Addis Ababa Abattoirs Enterprise (AAAE). Addis Ababa, the capital of Ethiopia, is located at 9.03° North latitude and 38.8° East longitudes with an average altitude of 2400 meters above sea level. The city covers about 54,000 hectares of land with an average population of more than 3 million. It has an average temperature during winter 6°c minimum and 23°C maximum and during summer 10°C minimum and 24°C maximum with an annual temperature of 15.9°C. It also receives an annual rainfall of 1089 mm with 60.1% annual relative humidity which ranges from 49% in February to 82% in July 14 (National Meteorology Service Agency, 2007)

Study animals

The study population covers all swine brought for slaughter from various parts of the country to Addis Ababa abattoir enterprise. Main sources of pigs in this study were from Addis Ababa, Bishoftu, Akaki and Shogollie. All pigs belonging to both sex (male and female) were considered for the purpose of this study. During the study period age was determined using tooth eruption patterns (Xiaolin, 2004) and swine were classified as young if they were less eleven month and adult if they were greater or equal to eleven month age (Bewuket et al., 2014).

Study design

A cross-sectional study was conducted from January 2018 to April 2018 in Addis Ababa abattoir enterprise to determine the prevalence of hydatid cyst infection in slaughtered pigs by using gross post mortem inspection procedure of visceral organs including liver, lungs, kidneys, and spleen.

Sampling method and sample size determination

Simple random sampling method was used for sampling selection. In this abattoir ante mortem and post mortem, examination was done by giving a special identification code for diagnosed animals so that this code made the sampling system easy i.e. lottery method of selection was held daily by using animals code until the end of the required sample size. The required sample size of the study for pigs was determined by the formula given in Thrusfield (2005) with 95% of confidence interval and at 5% desired precision. By using this formula and expected prevalence of 17% in pigs slaughtered in the study area, then the required sample size becomes 217. However, additional 34 samples were included as there was enough time to include extra samples which increases the precision of the study, and a total of 251 animals were examined for the presence of hydatid cysts in different organs like liver, lungs, kidneys and spleen for better conclusion.

$N=\frac{Z^2pq}{d^2}$; where N=number of animals to be sampled; P=expected prevalence, P=17%; d=desired absolute precision, d =0.05; the value of Z at 95% confidence interval= 1.96

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Prevalence study

The study was directed from January 2018 to April 2018. Ante mortem and postmortem examinations were carried out on 251 pigs that were slaughtered at AAAE.

Ante-Mortem examination

Physical examinations of the study animals were performed prior to slaughter in order to record data on age, sex and body condition. Age was determined using tooth eruption patterns (Xiaolin, 2004) and classified as young if it was less than or equal to eleven month and adult if it is more than eleven month (Bewuket et al., 2014). Origins of the pigs were also recorded. In this abattoir ante mortem and post mortem, examination was done by giving a special identification code.

Post-Mortem examination

During the postmortem examination, a thorough visual inspection, palpation and systematic incision of each visceral organ particularly the lung, liver, kidney and spleen was carried out according to procedures recommended by FAO (1994). The total number of hydatid cysts in each affected organ was noted to calculate the load of burden on organ and study animals' level. All hydatid cysts that found in the organs were collected to conduct cyst count and cyst size measurement.

Cyst number on infected organ

The total number of hydatid cysts in each affected organ was noted to calculate the load of burden on organ and study animal level. All hydatid cysts that found in the organs were collected to conduct cyst count and cyst size measurement. After counting the cyst burden on the examined organ, it was then classified as less than three cysts and more than or equal to three cyst. This classification was based on the frequency of cyst number on examined organ during the study period.

Cyst size measurement

Individual cyst diameter were measured using a ruler and classified into three groups as small, medium and large if the diameter of the cyst were <4 cm, 4– 8 cm and >8 cm, respectively. This measurement was provided in the same way with the study of Schantz (1990). Approximately similar measurement was used with the study of Endrias et al. (2010) in which all cysts in an organ were counted, then subjected to systematic size measurement (diameter) using a ruler and classified as small cyst (<3 cm), medium cyst (3-5 cm) and large cyst (>5 cm).

Annexe 1 - Picture of Picture of ante mortem examined pig

Annexe 2 - Picture of organ examined during post mortem examination

Annexe 3 - Picture of liver and lung infected with hydatid cyst

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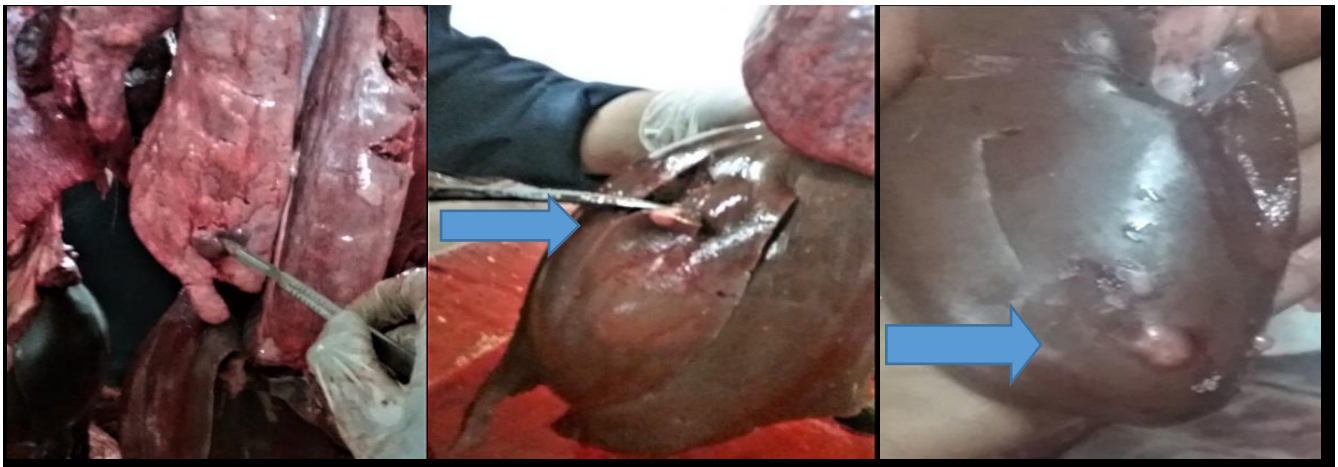

Annexe 4 - Picture of spleen and kidney infected with hydatid cyst

Data management and statistical analysis

The data collected was entered and scored in Microsoft excel worksheet. Before subjected to statistical analysis, the data was properly coded and thoroughly screened for errors. For analysis, STATA Microsoft software Version 14.1 was used. Descriptive statistical analysis such as frequencydistributions and cross-tabulations were used to summarize and present the data collected. Pearson chi square (χ^2) test was employed to assess the existence of association between prevalence of the hydatid cysts and different potential risk factors (age, sex, origin and body condition scores) considered. The total prevalence was calculated by dividing the number of hydatidosis positive animals by the total number of animals examined. For (χ^2) test, p- value<0.05 were considered significant whereas p-value>0.05 considered non-significant.

RESULTS AND DISCUSSION

The overall prevalence of hydatidosis

Among 251 heads of pigs slaughtered at Addis Ababa abattoir enterprise, 9.96% (25/251) were found infected with hydatid-cysts involving one or more different organs (Table 1). Rate of infection of hydatidosis in different age groups Young 12% (3/25) and Adult 88% (22/25) as well as body condition score with poor 40% (10/25), medium 32% (8/25) and good 28 % (7/25) were statistically significant (Table 2). Rate of infection of hydatidosis in different sex, male56% (14/25) and female 44% (11/25) with prevalence within sex of 10.94% (14/128) and 8.94% (11/123) respectively and also origin, Addis Ababa, Akaki, Bishoftu, Shegollie with prevalence within origin of 6.66% (3/45), 12% (3/25), 8.05% (12/149) and 21.87% (7/32) respectively were statistically not significant (Table 3).

The postmortem examination revealed that the distribution of hydatid cysts involved lung, liver, spleen and kidney. Among 25 pigs harboring hydatid cyst, 16(64%) of them had hydatid cyst infection only in a single organ whereas the remaining 9(36%) occurred in more than one organ with large proportion of pigs 9 (36%) had cysts only on lung and liver combination. With regard to single organ liver was the most affected organ 8 (32%) followed by lungs 5(20%), spleen 2(8%) and the least affected organ was the kidney which accounts only 1(4%) (Table 4).

With regard to cyst size, on 34 affected organs only lungs have large cyst while the remaining affected organs have small and medium cyst size. Liver had the highest percentage (44.1%) of small cyst. Spleen and kidney were the only organs that possess only small cyst size (Table 5). Except 4(11.8%) lungs which have more than or equal to three cyst number, all affected organs have distribution of less than three cysts number. Livers encompass highest percentage (50%) of less than three cyst number. During the postmortem examination higher percentage (88.2%) of organs were infested with less than three cyst burden whereas only 11.8% of cyst distribution on infected organ was with more than or equal to three cyst numbers (Table 6).

Table 1 - Total prevalence of affected pigs at Addis Ababa abattoir enterprise

Examined swine	Frequency	Percent	Cumulative
Affected pig	25	9.96	9.96
Non affected pig	226	90.04	100.00
Total	251	100.00	

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Table 2 - Prevalence of hydatid cyst with regard to age group and body condition score

Risk factor	No. of examined	No. of infected	Percentage	Prevalence	Total Prevalence	χ2	P<0.05
Young (<11 months)	92	3	3/25(12%)	3.26%	1.19%	7.2681	-
Adult (≥11 months	159	22	22/25(88 %)	13.83%	8.77%	-	0.007
Poor	113	10	10/25(40 %)	8.85%	3.99%	-	-
Medium	113	8	8/25(32 %)	7.10%	3.19%	10.273	0.006
Good	25	7	7/25(28 %)	28%	2.78%	-	-
Total	251	25	100%	9.96%	9.96%	-	-

Table 3 - Prevalence of hydatid cyst with regard to origin and sex

Risk factor	No. of examined	No. of infected	Percentage	Prevalence	Total Prevalence	χ2	P>0.05	
	Addis Ababa	45	3	3/25(12%)	6.66%	1.20%		-
Origin	Bishofu	149	12	12/25(48%)	8.05%	4.78%	-	-
	Shegolie	32	7	7/25(28%)	21.87%	2.79%	6.3297	0.09
Sex	Male	128	14	14/25(56%)	10.94%	5.57%	-	0.59
	Female	123	11	11/25(44%)	8.94%	4.38%	0.2782	
Total	251	25	100%	9.96%	9.96%	-	-	

Table 4 - Distribution of hydatid cyst in different organs

Organ	Number infected	Prevalence from infected animal	Prevalence from total examined animal
Lung & Liver	9	36%	3.59%
Liver	8	32%	3.19%
Lungs	5	20%	1.99%
Spleen	2	8%	0.80%
Kidney	1	4%	0.40%
Total	25	100%	9.96%

Table 5 -Distribution of hydatidcysts in different organs with respect to size

Organ	Small cyst	Medium cyst	Large cyst	Total
	Number with %	Number with %	Number with %	Number with %
Lungs	5 (14.7%)	5 (14.7%)	4 (11.8%)	14 (41.2%)
Liver	15 (44.1%)	2 (5.9)	0	17 (50%)
Spleen	2 (5.9%)	0	0	2 (5.9%)
Kidney	1 (2.9%)	0	0	1 (2.9%)
Total percent	23 (67.6%)	7 (20.6%)	4 (11.8%)	34 (100%)

Table 6 - Distribution of hydatidcysts with regard to number of cysts

Organ	Less than three cyst	More than or equal to three cyst	Total
	Number with %	Number with %	Number with %
Lung	10 (29.4%)	4 (11.8%)	14 (41.2%)
Liver	17 (50%)	0	17 (50%)
Spleen	2 (5.9%)	0	2 (5.9%)
Kidney	1 (2.9%)	0	1 (2.9%)
Total	30 (88.2)	4 (11.8%)	34(100%)

DISCUSSION

The present study found that the prevalence of hydatidosis in pig was 9.96%, which can be regarded as high on what grounds? Provide a citation of a similar study that reported so. This high prevalence may generally relate to the presence of favorable factors for the transmission and maintenance of high level infection in the areas. This finding is in agreement with other prevalence studies in Africa; 19% by Brahmi (1973), 5% by Dada et al. (1979), 0.7% by Larbaoni et al. (1980), 0.9% by Gathura et al. (1988), 4.6% by Rahman et al. (1992), and 16.9% by Bewuket et al. (2014), in Tunisia, Nigeria, Algeria, Kenya, Egypt, and Ethiopia respectively).

Unlike the results of the current study, Bewuket et al. (2014) recorded a higher prevalence of 16.9% (65/384) in Addis Ababa abattoir enterprise. This variation could have arisen due to the fact that Bewuket's data was gathered from different region of the country and his study period was higher than the current finding. This may be because in the past dogs were often kept close to intermediate hosts. On the contrary, such practices occur to a much lower degree these days due to enforcement of veterinary guideline. According to Garippa et al. (2004), variation in prevalence of hydatidosis from one area to other area could result from dissimilarities in animal husbandry systems like free grazing, uncorrected

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slaughter of animals, lack of proper removal of infectious carcass and the presence of more number of stray dog and their associations with animals. When compared to other countries in the world, varying prevalence of cystic echinococcosis have been reported in swine by several researchers. This finding (9.96%) is lower than that of Lidetu and Hutchinson (2007) studies (31.1%) in north Queensland. These researchers have done on the feral pigs. The result in this study might indicate that the prevalence of hydatidosis in pigs slaughtered at Addis Ababa abattoir enterprise has a low chance of getting the disease from dogs' compared to those of feral pigs. This might be because of dogs were highly contacting and the way they were feeding with feral pigs during hunting season; thus may contribute to the maintenance and raises of hydatid disease prevalence in feral pigs than the present study. Even though, degree of infection is less than that of feral pig there is the chance of getting the hydatiddiseases from dogs to different swine farms and abattoirs.

Livers and lungs were the most frequently infected visceral organs inspected. In the present study among pigs harboring hydatid cyst, 16(64%) of them had hydatid cyst infection only in a single organ whereas the remaining 9(36%) occurred in more than one organ with large proportion of pigs 9 (36%) had cysts only on lungs & liver combination. With regard to single organ, liver was the most affected organ 8 (32%) followed by lungs 5(20%), spleen 2(8%) and the least affected organ was the kidney which accounts only 1(4 %). This finding is in agreement with the literature that revealed hydatid cyst is most commonly found in liver and lung of ungulates (Hubbert et al., 1975). This could be reasonable by the fact that livers and lungs have the first great capillaries sites encountered by the migrating Echinococcus oncosphere (hexacanth embryo) which adopt the portal vein route and primarily negotiate hepatic and pulmonary filtering system sequentially before any other peripheral organ is involved (Kebede et al., 2009). During the study period spleen and kidney were the least affected organs in the examined pigs. This finding is in agreement with the study that indicated the liver and lungs are the most commonly affected organs with hydatid cyst. However development of hydatid cysts occurs occasionally in other organs and tissue when oncosphere escape in to the general systemic circulation (Urquhart et al., 1996).

In the current study, cysts were found more in liver (6.78%) than lungs (5.58%). The findings conform to those reported in a similar study by Bewuket et al. (2014) in which the liver was the highest infected organ than any other organs/tissues. The liver being the most commonly affected organ than any other organ might be due to the reflection of the route of parasite entry and seem to support the hypothesis of hepatic portal distribution of oncosphere leading firstly to liver infection (Schwabe, 1986). This also might be justified by the fact that pigs are slaughtered at adult age in Addis Ababa abattoir enterprise. During older ages, the liver capillaries were dilated and most oncosphere pass directly and used for hexacanth embryo to enter the lymphatic circulation at last that can be carried through the thoracic duct to the lungs (Bekele and Butako, 2010). This may decrease the prevalence of the occurrence of hydatid cysts in swine lungs, kidney and spleen during the study period.

During the study time single and multiple hydatid cyst distribution were recorded in different organs. Out of 34 infected organs, 14.7% and 11.8% of lungs had medium and large-sized cysts respectively while the liver (44.1%) harbored higher number of small sized cysts. This finding is in agreement with the study of Larrieu et al. (2001) in which higher percentage of medium and large cysts in the lungs may be related with the softer consistency of the lung while the higher yield of calcified cysts in liver could be attributed to relatively higher reticulo endothelial cells and abundant connective tissue reaction of the organ. High proportion of small cysts on liver may be due to immunological response of the host which might preclude expansion of cyst size (Torgerson, 2002).

During the study period 30 (88.2%) infected organs have less than three cyst count while only 4 (11.8%) infected lungs have more than or equal to three cyst count. In this study liver have highest percentage (50%) of less than three hydatid cyst count followed by lung (29.4%), spleen (5.9%) and kidney (2.9%). Such variations in cyst abundance might be due to the spatial distribution and the infectivity of *E. granulosus*eggs and the susceptibility and defensive capabilities of the host that agree with the study of Macpherson et al. (1985).

The present finding revealed there was no statistical variation in the prevalence rates between the areas where the examined animals comes from (Bishoftu, Akaki, Shegolie and Addis Ababa). The reason for the absence of variation in the prevalence in those different places may be related with the presence of very similar environmental situation in all the four areas. However, other investigators Lidetu and Hutchinson, (2007) and Bewuket et al. (2014) found a variation in the prevalence's of swine hydatidosis for different areas having different environmental and climatic conditions, stocking rate and the abundance of infected definitive and other intermediate hosts. Other factors may be due to difference in feeding habits, social activity and attitude of peoples in difference region (Azlaf and Dakar, 2006) and difference in strains of *E. granulosus* that exist in different geographical location (Parijia, 2004).

Analysis of body condition of swine had significant association with the occurrence of the hydatid disease and swine with poor body condition were with higher prevalence of hydatidosis. The difference between bodies conditions score may be because swine with poor body conditions have low immunity to hydatidosis. This finding was in agreement with the result of Zelalem et al. (2012). And also Polydorous, 1981 reported moderate to severe infection of the parasite may cause retarded growth and weight loss. Bewuket et al. (2014) reported different results as the body condition had no significantly influence on the prevalence of hydatidosis.

There was significant variation ($P<0.05$) in swine with different age groups where adult pigs were highly infected with the prevalence within age of 13.83% and young pig have 3.26% prevalence. This result is in consistence with

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Bewuket et al. (2014) in which there was statistically significant variation (P<0.05) in swine with different age groups where adult were highly infected with the prevalence of 23.55 % and young pigs have 3.2 % prevalence

CONCLUSION

In conclusion, the findings in this study showed that the prevalence of hydatidosis in pigs slaughtered at Addis Ababa abattoir enterprise was 9.96%. Age and body condition were risk factors for hydatidosis infection. Among infected organs with hydatidosis, only lungs have large cyst size while the remaining affected organs have small and medium cyst size. Liver have highest percentage of small cyst. Spleen and kidney were the only organs that possess only small cyst size. Except lungs which have more than or equal to three cyst number, all affected organs have a distribution of less than three cysts number. In case of cyst number, higher percentage of organs was infested with less than three cysts. Livers encompass highest percentage of less than three cyst number. This result indicated that hydatidosis was a significant disease that disturbs swine. Based on the above conclusion the following recommendation is forwarded: 1) A serious farm to table swine health management should be advocated; 2) Rearing of swine by dividing in to their age and body condition should be advised for swine farm owners; 3) Educating of the society about hydatidosis life cycle and its consequence should be done. Further research should be conducted to study the potential sources of hydatidosis for effective prevention and analysis of its economic impact.

DECLARATIONS

Authors' contributions

MB conceived the study, coordinated the overall activity, and carried out the statistical analysis, drafted the manuscript and participated in the design of the study, and reviewed the manuscript. All authors read and approved the final manuscript. AY participated in drafting and reviewing the manuscript. EG conceived the study, coordinated the overall activity, and reviewed the manuscript and participated in drafting and reviewing the manuscript.

Availability of data and materials

Data will be made available up on request of the primary author.

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A REVIEW ON CHEMICAL CASTRATION METHODS TO CONTROL STRAY DOG POPULATION

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ABSTRACT: About 75% of dogs worldwide are free to roam and reproduce, thus creating locally overabundant populations. Problems caused by roaming dogs include diseases transmission to livestock and humans, predation on livestock, attacks on humans, road traffic accidents, and nuisance behavior. Nonsurgical fertility control is increasingly advocated as more cost-effective than surgical sterilization to manage dog populations and their impact. The aim of this review was to illustrate the spectrum of fertility inhibitors available for dogs. Although surgery is the most effective and safe procedure, it is also expensive to use of non-surgical, sterilization methods that would make male sterilization inexpensive, easy and fast for sterilization of large number of male dogs within short period of time to effectively contribute in curbing the growth of the stray dog population were introduced. Chemical sterilization methods so far employed included hormonal methods, immunocontraceptives and ınorganic chemo-sterilants (chemo-sterilants such as $CaCl_2$, zinc gluconate neutralized by arginine (Neutersol) and hypertonic sodium chloride-NaCl solution. Intratesticular injection of calcium chloride, Zinc gluconate and 20% NaCl hypertonic solution showed a promising result as chemical sterilants. The review concluded that the main challenges for the future are evaluating the feasibility, effectiveness, sustainability, and effects of mass non-surgical sterilization campaigns on dog population size and impact as well as integrating nonsurgical fertility control with disease vaccination and public education programs. The review also showed the relative lack of research or knowledge related to fertility inhibitors in developing country as Ethiopia and suggested that more works is required in this country.

Keywords: Chemical sterilization, Dog population management, Fertility inhibitors, Stray Dogs.

INTRODUCTION

Dogs are one of the primitive companion animals to be domesticated by man. Their domestication dated as far back as 8,000 BC (Matznick-Koler, 2002). Street dogs are common in the developing world, and often live with little or no veterinary care, consuming refuse and feces to survive. The uncontrolled growth of a dog population can have a negative impact on public health and can create socioeconomic, political, and animal welfare problems (OIE, 2016). The global dog population is estimated to be around 700 million (Hughes and Macdonald, 2013). Problems caused by free-roaming dogs include diseases transmitted to livestock and humans, predation on livestock, bites, road traffic accidents, and nuisance such as barking and soiling of parks and recreational areas (Macpherson et al., 2013).

Domestic dogs are successful breeders in almost all kinds of environments, but are always directly or indirectly dependent on man. Since their domestication more than 14 thousand years ago a strong link between dogs and humans has been established. Nowadays that link persists even stronger, since the affection for and loving care given to this species has generated a tremendous industry devoted to caring for its health, nutrition, training, etc. However, as a consequence of uncontrolled reproduction, overpopulation of this species (probably the most abundant carnivore species on earth) has become a big concern in many countries where stray and feral dogs are causing zoonotic diseases, nuisance, pollution of parks and recreation areas, damage to livestock and destruction of ecosystems. In addition, the relinquishing of dogs to animal shelters incurs great costs for the maintenance or euthanasia of dogs because not enough households are available for their re-homing (FAO, 2014).

Dogs play a number of important roles in human societies: they provide companionship and are used for a variety of activities including hunting, herding other animals and guarding property. Animals live in close contact with human beings. In many countries, however, an increasing number of unwanted, unhealthy and unvaccinated dogs are found roaming. This is especially the case in countries with limited social and economic development as well as in places where civil unrest or armed conflict have forced people to flee from their homes and leave their dogs behind. Abandoned and free-roaming dogs can give rise to a series of human and animal health and welfare concerns, in urban spaces and in other human habitats. The availability of more food waste, due to changes in society such as urbanization and increased human densities, combined with a lack of responsible ownership, are leading to an apparent increase of free-roaming dogs (Pal, 2005).

National and international organizations working on dog population management and welfare often classify dogs in the following categories, according to ownership and degree of confinement: owned and permanently confined within household premises; 2) owned by a single household but free to roam; 3) "community owned," with several households or people providing food and shelter but free to roam; or 4) ownerless and free-roaming. As a consequence of uncontrolled reproduction, canine overpopulation remains a problem facing many countries throughout the world, where it creates locally overabundant populations of animals that are often in poor health and have a high turnover because of low survival rates.Approximately 75% of the global dog population is free roaming or stray dogs, living mostly in Latin America, Africa and Asia (Matter and Daniels, 2000).

The need to control the number of dogs, especially stray dogs, is motivated in part by public health concerns, particularly in relation to rabies transmission. Additionally, echinococcosis/hydatidosis and leishmaniasis are serious zoonotic diseases transmitted by dogs (ICAM, 2007).

There are two techniques for sterilization known as surgical and chemical. Surgical castration removes the testes from the scrotum via an incision in male animals. This method is effective, but infection or bleeding can become a problem and it is also time consuming, not cost-effective and needs skilled surgeons. Moreover, this method is not effective for large-scale application, especially for controlling large population size of undesirable mammals in the community like stray dogs. Besides this, postoperative care and management of the animal are also required to prevent infection (Jana et al., 2007).

The use of inorganic chemo-sterilants in male dogs is an attractive option because it removes the disadvantagesand costs of surgical sterilization and post-operative care (Rojas et al., 2011). Furthermore, in regions where surgical castration of male dogs is not culturally accepted (e.g. Romania, the Bahamas) chemical castration offers a reasonable alternative. Hence, chemical castration could be an attractive option for developing countries with limited resources for surgical dog management programs (Garde et al., 2016). Surgical castration has disadvantages and postoperative complications of surgical methods of sterilization such as hemorrhage, wound dehiscence, infections, and scrotal swellings (Adin, 2011). In order to minimize post-operative complications and costs associated with conventional surgical castration, other in situ noninvasive approaches have been used which include immune-castration and chemical castration (Abshenas et al., 2013).

The use of non-surgical, inexpensive and easy sterilization methods of a large number of male dogs would effectively contribute to curb the growth of the pet population. Alternative methods to surgical sterilization that are effective, easy to administer, safe, and affordable would offer immense benefits, allowing animal welfare organizations, public health programs, and governments to reach further with limited resources (Briggs, 2012). Immunocastration works as a vaccine, stimulating the immune system to produce antibodies against the gonadotropin-releasing hormone (GnRH) (Thompson, 2000). About 99% of rabies deaths occur in developing countries 55% in Asia and 44% in Africa. Rabies mortality ranges from 0.001 per hundred thousand in the United States to 18 per hundred thousand in Ethiopia, with mortality levels of 0.01 in South Africa, 0.47 in Thailand and Vietnam, 0.57 in Sri Lanka, 1.75 in Bangalesh, and 2–4 in India (Haupt, 1999).

The ideal non-surgical castration technique should produce permanent loss of fertility, permanent loss of sexual behaviour including displays of some forms of aggressive behaviour, requires single injection, safe, with no deleterious side effects for the target and non-target species (including humans) in case of accidental exposure or self-injection, has good efficacy (high success rate in treated animals), technically feasible, stable in formulation, allow for storage and handling under field conditions and should be affordable and cost effective (Kutzler and Wood, 2006).

Calcium chloride ($CaCl_2$), delivered as intratesticular injection, is being researched as a sterilant for dogs. $CaCl_2$ caused atrophy of the seminiferous tubules and decreased testosterone concentration and sperm count in a dose dependent manner (Jana and Samanta, 2007). The low cost, ease of use, and cultural acceptance of a sterilization method that does not require removal of the testes make male sterilants a valuable tool for large-scale sterilization campaigns, particularly in areas lacking clinical facilities or skilled staff (Levy and Crawford, 2008). Sodium Chloride Solution - Hypertonic saline is a solution that is inexpensive and easy to administrate. Hence, chemical castration could be an attractive option for developing countries with limited resources for surgical dog management programs (Garde et al., 2016).

In Ethiopia Stray dog population spreads several zoonotic diseases majorly rabies. Dog population risk in Ethiopia by rabies the national annual estimates from official reports indicate 12 exposure cases per 100,000 population and 1.6 rabies deaths per 100,000 population (Deressa et al., 2013). However, the actual numbers are expected to be higher as many cases are not reported (Moges, 2015). Rabies is an endemic disease in Ethiopia claiming thousands of lives each year. It is a serious challenge in Tigray region, Northern Ethiopia, both in humans and animals. A report from Tigray Bureau of Health indicates an estimated 4729 dog bite cases and 44 deaths from 2009-2012 in the region (TBoH, 2012).

Therefore the main objectives of this review are: The potential uses of non- surgical castration methods to control stray dog population; to reduce the cost of surgery and complications; and to give updated information about chemical castration other than surgical intervention

LITERATURE REVIEW

Dog classification

Populations of dogs vary between different habitats, different cultures and different social strata of the human populations. In developing countries, a large number of free roaming dogs have owners and are classified according to the

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WHO (1990) based on the level of restriction or supervision as family dogs (fully dependent; semi-restricted) which can have access to the streets, and neighborhood dogs (semi-dependent, semi-restricted or unrestricted). The presence and abundance of stray dogs depends on the attitudes towards dogs of the humans in these areas. Stray dogs have a great impact on human public health and the ecosystems. Due to the close contact with humans, dogs are responsible for the spread of several zoonotic diseases. Dog rabies is endemic in many developing counties and is responsible for most human deaths from the disease. In addition, at least 65 other zoonosis including ancylostomiasis, echinococcosis, leptospirosis and salmonellosis, may be transmitted to man by direct contact or contact with secretions and excretions of pets. Additionally, domestic dogs are potentially effective predators of the native fauna and can have competitive interactions with endemic wild carnivores (Butler et al., 2004).

Strategies to control the overpopulation of free-roaming dogs include enforcement of law, education of owners and sterilization of pets. In many developing countries, mass euthanasia of dogs is systematically-used in an effort to reduce the density of free-roaming dogs and prevent the transmission of zoonotic diseases. However, this strategy cannot be effective in the long term without the enforcement of laws and education of people. Free-ranging domestic dogs are non-cooperative populations, i.e. they are not dependent on other animals of the same species to survive. Any reduction in the population density through additional mortality is rapidly compensated by better reproduction and survival. In a hypothetical model adding mortality (a) at different magnitude and frequency to two different kinds of populations, non-cooperators (b) and cooperators (c) shows than non-cooperator breeding populations can recover quickly even from important or frequent perturbations (Courchamp et al., 1999).

The most important canine zoonosis includes rabies, leptospirosis, Chagas disease, echinococcosis, and leishmaniasis (Garde et al., 2013). Rabies is caused by fatal encephalitis in most mammals including humans. Animals like dogs, bats, raccoons, skunks and foxes act as reservoirs and the virus is transmitted through bites and licking. The incubation period can vary between 2 weeks and several years, with an average of 2-3 months (WHO, 2005).

DISCUSSION

Methods of chemical contraception and chemical sterilant

Reproduction control utilizing chemical or immunological methods offers a humane and less expensive alternative to surgical sterilization (FAO, 2014). Contraception in dogs can be achieved through chemical reproductive control, which prevents pregnancy by temporarily or permanently sterilizing these animals (Kutzler and Wood, 2006). Chemical fertility control can be achieved through contraception, which prevents the birth offspring but maintains fertility or by sterilization, which renders animals infertile (Kutzler and Wood, 2006). Chemical sterilization methods so far employed include hormonal methods, immunocontraceptives and Inorganic chemo-sterilants such as CaCl2, zinc gluconate neutralized by arginine (Neutersol), NaCl solution (Kutzler and Wood, 2006).

Hormonal methods. Gonadotropin-releasing hormone (GnRH) is a decapeptide synthesized and stored in GnRH neurons located There have been three isoforms of GnRH recognized in animals; mammalian GnRH-I, chicken GnRH-II, and lamprey GnRH-III (Khan et al., 2007). However, GnRH-I is accepted as the main fertility-regulating peptide (Khan et al., 2007). Release of GnRH is controlled by steroidal hormone feedback as well as non-steroidal hormones, such as melatonin, catecholamines and opioids. GnRH is one target for fertility inhibitors. GnRH controls the release of the pituitary gonadotropins, Follicle stimulating hormone (FSH) and Luteinizing hormone (LH), which in turn control the production of sex hormones and ultimately ovulation, spermatogenesis, and sexual behavior (Massei and Miller, 2013). The working mechanism of these agents consists in binding to GnRH receptors and therefore stopping the action of endogenous GnRH. This suppresses gonadotropins secretion and blocks the ovulation without initial stimulatory effect. When given to pregnant bitches it can induce abortion which is not the case in queen. In 2006 an implant containing nafarelin at a dose of 18.5 mg, called Gonazon, was introduced on the European market. This compound is inserted in the umbilical region in dogs, and in the neck in cats (Gobello, 2012). Another formulation of GnRH agonist is marketed as the implant Suprelorin comprising of a GnRH analogue, named deslorelin. Its activity is similar to what is described above, but currently it is only registered for temporary chemical castration of male dogs. Off-label use has shown that the drug can also be used for long-term prevention of heat in female dogs. However, frequently an initial heat is observed shortly after the implant is inserted due to the flare-up effect (Fontaine and Fontbonne, 2011).

Androgens. Preparations of this group are widely used for female contraception. The use of injectable or oral testosterone derivatives prevents oestrus in females. The synthetic androgen mibolerone exists in the form of a commercial oral preparation (Cheque Drops) for dogs and cats in the United States (Eade et al., 2009). The formulation is characterized by anabolic and antigonadotropic activity of the hormone. If the treatment is implemented at least 30 days before the start of a pro-oestrus, the heat is suppressed. The treatment can be continued for two years. Longer administration is not recommended because of possible hepatotoxicity. After discontinuing, the subsequent oestrus occurs in a period of 1 to 7 months with an average of 70 days (Kutzler and Wood, 2006).

Immunocontraception. For a long time attempts are exerted to use immunological phenomena for contraception in animals. The main idea consists in the induction of antibodies directed against antigens playing an important role in reproduction. An antigen– antibody reaction should lead to infertility lasting as long as a sufficiently high level of specific

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antibodies exists in the blood circulation. The main problem however is the choice of suitable antigen characterized by adequate immunogenicity in order to achieve a contraceptive vaccine (Munks, 2012).

Zona-pellucida antigens. The zona-pellucida is a non-cellular glycoprotein membrane covering the oocyte and (after fertilization) the embryo up to the late blastocyst stage, whereupon it brakes and disappears. Three major zonal glycoproteins with different molecular weight designated as ZP1, ZP2 and ZP3 have been isolated from the membrane. Apart from its structural function the zona-pellucida is of great functional importance. It contains sperm receptor sites, which allow fertilization. Antizonal antibodies do not allow fertilization, without affecting the hormonal activity. Experiments were conducted in dogs and cats by using both allogeneic or xenogeneic zonal antigens, in particular derived from swine oocytes, which are easy to obtain because of wide availability of fresh slaughter material. Also recombinant antigens, obtained by biotechnological methods were used (Eade et al., 2009).

Inorganic Chemostrilant

A) Zinc gluconate neutralized with arginine (zeuterin): This formulation of the chemical compound of zinc gluconate neutralized with arginine was developed to chemically sterilize male dogs. The formulation was initially developed by Pet Healthcare International; it received approval from the United States Food & Drug Administration (FDA) in 2003 and was distributed in the U.S. under the name Neutersol® until Early 2005. Zeuterin™ is a non-surgical sterilant for male dogs delivered via intratesticular injection. The ideal method of chemical sterilization needs to meet three key criteria to be regarded as a good alternative tosurgical sterilization. First, it has to be effective in a high percentage of treated animals. Second, it should have ahigh margin of safety, without adverse effects for the environment. Third, it has to be permanent and irreversiblefollowing a single treatment. The first product obviously fulfilling these criteria was zinc gluconate. It was firstdescribed by Fahim et al. (1993). Zinc gluconate was provided as a sterile aqueous solution containing 0.2 M zinc gluconate (13.1 mg/mL) neutralized to pH 7.0 with 0.2M l-arginine in glass vials containing 2 mL of readyto-use solution.c The drug was supplied with plastic calipers and instructions for measuring the width of each testicle; a dosing chart was used to correlate testicular width (10 to 27 mm) with volume of zinc gluconate (0.2 to 1.0 mL). In this project, dogs of all ages were treated by following the product label recommendation for volume of injection. Dogs with testes measuring < 10 mm in width received zinc gluconate volumes of 0.1 mL/testicle. Volumes for dogs with testes measuring > 27 mm in width were truncated at 1.0 to 1.1 mL/testicle (1.0 mL is the highest approved dose). The active ingredient is zinc gluconate neutralized with arginine. The formulation causes permanent sterility in one treatment, the process of neutering with Zeuterin is also known as “zinc neutering. the treatment with this chemical does not require general anesthesia, sedation is recommended to prevent movements of the dog during injection (Kutzler and Wood, 2006). Correct injection technique was foundcritical for the safe use of Neutersol in order to avoid ulceration of the scrotum and painful swelling of the testes (Kutzler and Wood, 2006).

The exact mechanism of action of Zeuterin is not known. The product is administered as an intratesticular injection into the center of the testicle via the dorsal cranial portion of testicle, parallel to the longitudinal axis. After injection, the compound diffuses in all directions from the center of the testis. In the concentration used, zinc gluconate acts as a spermicide and destroys spermatozoa in all stages of development and maturation (Massei and Miller, 2013). Zinc gluconate is absorbed and metabolized by the body within 72 hours after the injection. As the dog’s body increases blood flow and creates inflammation to heal, it results inpermanent and irreversible fibrosis in the seminiferous tubules, rete testis, and epididymis. This process results in permanent sterilization, and the endocrine feedback system remains intact. Following injection, the testicles atrophy over a period of time ranging from weeks to months, resulting in a reduction in testicular size and changes in shape or texture. These changes may or may not be symmetrical.Hence, chemical castrationcould be an attractive option for developing countries with limited resources for surgical dog management programs (Garde et al., 2016).

B) Calcium chloride ($cacl_2$): Nonsurgical male sterilization techniques have been evaluated as a means to avoid the potential health complications, expense, expertise and facilities required for surgical sterilization procedures. One of the most promising is calcium chloride (CaCl2), which has been utilized to chemically castrate a variety of species Calcium chloride (CaCl2), delivered as intratesticular injection, is being researched as Inorganic Chemo-sterilants for dogs (Massei and Miller, 2013). Following intratesticular injection of $CaCl_2$, necrosis, fibrosis and degeneration of seminiferous tubules and Leydig cells occurs, reducing or eliminating the production of spermatozoa, testosterone and sperm counts in a dose-dependent manner in male dogs (Jana and Samanta, 2007). Calcium chloride dihydrate ($CaCl_2$) has been the subject of renewed interest as a potential injectable sterilizing agent for male dogs and cats that may reduce testosterone levels more significantly, and might carry less risk of severe injection-site reactions, than other injected sterilizing agents. Additionally, $CaCl_2$ has spurred discussion because it can be made from readily available ingredients, raising questions about the legality, ethics, appropriateness, and advisability of its use in the many countries in which Zeuterin/EsterilSol is not available and is not projected to come to market in the immediate future. $CaCl_2$ was effective and economical for the sterilization of male dogs. It is free from pain and chronic stress and will contribute to a simple alternative (Jana and Samanta, 2007).

C) $CaCl_2$ formulation and dosing chart: The alcohol solution of 20% calcium chloride dihydrate is prepared by the veterinarian or by an accredited compounder. Prepare as follows: Formulation: 20 gr of pharmaceutical grade $CaCl_2$ (dihydrate) is brought to a final volume of 100 ml of 95% pharmaceutical grade ethanol, mixed, sterilized by autoclave or syringe filter and delivered in a stopper top container (Jana and Samanta, 2007). Always pull 0.2 ml of calcium chloride

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over the maximum recommended dose. Up to 10% of dogs require up to 0.2 ml more calcium chloride in order to achieve a firm feeling upon injection. This includes large and small dogsas elongation of the testicle may change the required volume significantly (Oliveira et al., 2012). Supplies: Calcium chloride, Caliper, Luer lock syringes with 1" 23 gauge needles, Separate 1 ½" 23 gauge needles, Gauze in chlorhexidine solution mixedaccording to manufacturer's directions, Drugs for sedation,Ketophen or other pain management,Exam gloves if desired (Oliveira et al., 2012).

Chemo sterilization is hypothesized to result from edema that follows intratesticular injection of CaCl2, leading to necrosis and fibrosis and degeneration of seminiferous tubules (and germ cells) and the interstitial (Leydig) cells (Jana and Samanta, 2007). As testosterone concentrations fall, the integrity of the seminiferous tubule is further compromised. Have also proposed a role for free radicals in the mechanism of action of CaCl2 injection. According to this hypothesis, CaCl2 causes production of free radicals in testicular tissue, leading to lipid peroxidation and destruction of cellular structures, and also directly impairing spermatogenesis and androgenesis (Soumendra and Shyamal, 2017). Studies from this group have demonstrated decreased activities of catalase, glutathione peroxidase, glutathione S-transferase, and superoxide dismutase, decreased levels of reduced glutathione, and increased levels of conjugated dienes, with increased doses of CaCl2 (Jana, 2011).

Table 1 - Dosage and testicular width with their dose per testicle

Testicular Width	Dose per testicle
10-14 mm and sexually mature adult cats	0.25 ml (if testis feels overly full, STOP before full dose)
15-18 mm	0.5 ml
19-22 mm	0.8 ml to 1 ml (continue to fullness)
23-24 mm	1 ml to 1.5 ml (continue to fullness)
25-26 mm	1.5 ml to 2 ml (continue to fullness)
27 mm and above	1.5 ml to 2.5 ml (continue to fullness)

Source: www.calciumchloridecastration.com/chemical-castration-instructions-for-veterinarians

Hypertonic Sodium Chloride (NaCl)

Sodium Chloride Solution - hypertonic saline is a solution that is inexpensive and easy to administrate and revealed that severe degenerative changes in testicular seminiferous tubules and massive infiltration of immune cells in hypertonic saline group. Additionally researchers indicated that "intratesticular hypertonic saline injection seems to be an alternative method in the future to its rivals such as orchiectomy and medical castration" but that further laboratory work would be required to ascertain the potential utility of this approach in dogs (Emir et al., 2008). In other study, it was observed that 20% sodium chloride could be used for chemical castration in young dogs. It was suggested that intra testicular injection of hypertonic saline could be an effective method for nonsurgical sterilization of the non-adult male dogs but not adult dogs (Ibrahim et al., 2016). The hypertonic solution was prepared by dissolving NaCl (200 mg/mL) in ultrapure water. After dilution, the 20% NaCl solution was autoclaved in 50 mL glass flasks and stored at 5 C until use. The use of inorganic chemo-sterilants in male dogs is an attractive option because it removes the disadvantages and costs of surgical sterilization and post-operative care (Rojas et al., 2011). Furthermore, in regions where surgical castration of male dogs is not culturally accepted (e.g. Romania, the Bahamas) chemical castration offers a reasonable alternative (Garde et al., 2016). Hence, chemical castration could be an attractive option for developing countries with limited resources for surgical dog management programs (Garde et al., 2016).

Advantage and disadvantage of surgical Castration methods

A) Advantage of surgical castration methods: Surgical sterilization of dogs is one of the most commonly performed procedures in veterinary practice, and is done as a method of contraception to aid in the pet overpopulation problem, as well as to prevent diseases of reproductive system, such as benign prostatic hyperplasia and to modify undesirable behavior, such as internal aggression and mounting of other dogs, Will not be able to reproduce, will not get ovarian or uterine cancer, Will not have dangerous uterine infections, Will not mark territory by urinating or spraying, lessens tendency to fight with other animals despite castration is almost the sole method for control of pet's overpopulation globally.

B) Disadvantages and postoperative complications: For many years, surgical castration has been considered a standard gold tool for sterilization of male animals. However, several drawbacks have been associated with this procedure such as high cost, time consumption, need for postoperative care and management, risk of post-operative complications, small-scale application, the requirement of anesthesia, medical equipment, a sterile surgical suite, a trained veterinarian, recovery time, and incision site observation (Jana and Samanta, 2007). Surgical castration also do have complication such as, hemorrhage, wound dehiscence, infections, and scrotal swellings, requires anesthesia has some morbidity and mortality and expensive and technical also not available in much of world (Adin, 2011).

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Advantages and disadvantage of non- surgical castration methods

A) Advantage of non-surgical castration methods: An ideal chemical sterilizing agent would be one that effectively arrests spermatogenesis and androgenesis as well as libido with absence of toxic or other side effects (Wiebe et al., 1989). Advantages of chemical castration are apparent reduction in pain and stress as well as elimination of hemorrhage, hernia, infection, myiasis and other surgical sequela. It is also suited for mass-scale sterilization, simple and inexpensive (Ibrahim et al., 2016). This method may offer savings in cost, time, and facility requirements, thus helping animal welfare organizations sterilize more animals and/or redirect resources to other lifesaving projects. It also presents an option for pet owners who would prefer to sterilize their dog without surgery. The low cost, ease of use, and cultural acceptance of a sterilization method that does not require removal of the testes make inorganic chemo-sterilants a valuable tool for large-scale sterilization campaigns, particularly in areas lacking clinical facilities or skilled staff (Levy et al., 2008).

B) Disadvantage of non-surgical castration methods: As a result of these chemical injections, side effects have been documented. The dog will experience pain in his scrotum for three to five days after the injections. There was swelling, redness, and irritation. Lethargy and diarrhea are known side effects that are usually temporary (Threlfall and Immegart, 2000).

CONCLUSIONS AND RECOMMENDATIONS

The use of fertility inhibitors is gaining acceptance to control populations of companion animals and wildlife. For dog population management, nonsurgical sterilization is increasingly advocated as deserving priority for development because of its potential to be more costeffective than surgical sterilization. For dog population management, nonsurgical or chemical sterilization is increasingly advocated as deserving priority for development because of its potential to be more cost effective than surgical sterilization. Chemical sterilization methods so far employed include hormonal methods, immunocontraceptives and Inorganic Chemo-sterilants such as calcium chloride-$CaCl_2$, zinc gluconate neutralized by arginine (Neutersol), sodium chloride-NaCl solution). This review indicated that the past decade saw a significant increase in studies concerning fertility inhibitors for dogs. If nonsurgical fertility control is chosen to manage dog populations or their impact, social acceptance, humaneness, effectiveness, feasibility, costs, and sustainability of this method should be evaluated at an early planning stage. This framework is based on the assumption that a set reduction of dog population size or the elimination of a disease such as rabies, within a predefined timeframe can be achieved by using nonsurgical fertility control as an additional tool to education and vaccination.

The trend of using calcium chloride should be adopted to the community; training should be given to the practitioners in the way how to inject the chemical into the testicle; the government should take into consideration in policy making in the application of calcium chloride for dog population control; further research should be conducted to reduce side effects of calcium chloride.

DECLARATIONS

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Dr. Addisu Mohammed Seid and Demr Abebe Terefe equally participated in the reviewing of this paper. We reviewed the paper and contributed in developing the content.

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LEVELS OF AFLATOXINS AND FUMONISINS IN POULTRY FEED FROM GHANA

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Supporting Information

ABSTRACT: Mycotoxins are secondary fungal metabolites that contaminate animal feeds, crops and food. Globally, two major foodborne mycotoxins (aflatoxins and fumonisins) have been reported to affect the health and productivity of the poultry industry. Notwithstanding the health risks associated with these mycotoxins, no study has probably investigated the co-occurrence of aflatoxin and fumonisin levels in poultry feed produced in Ghana. The aim of the study was to investigate the levels of total aflatoxin (B1, B2, and G1 and G2) and fumonisin B1 (FB_1) levels in poultry feed produced in Ghana. Total aflatoxins and fumonisin B1 were analyzed in 100 poultry feed samples collected from farmers from four major poultry producing regions in Ghana. High performance liquid chromatography (HPLC) was used to measure total aflatoxin levels whiles a fluorometer reader was used to measure the levels of fumonisin B1. Total aflatoxin and FB_1 contaminations were detected in 100% of the feed samples in a range of 0.02-22 ppb and 0.5-4.6 ppm, respectively. Three samples representing, 3% out of the 100 samples screened were detected to have total aflatoxin levels greater than 20 parts per billion Fumonisin levels detected in the poultry feed were within the permissible level of 50 parts per million in poultry feed. Seventy out of the 100 poultry feed samples collected had their feed treated with fungal binders. In present study, we have probably for the first time in Ghana shown the levels of mycotoxins in poultry feed and the need to monitor animal feed made from cereals.

Keywords: Poultry feed, Total aflatoxin, Fumonisin B1, Ghana

RESEARCH ARTICLE

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INTRODUCTION

Mycotoxins (aflatoxin and fumonisin) are toxic secondary metabolites produced by certain fungi in agricultural products that are susceptible to mould infestation. Mycotoxin contamination is difficult to predict, which makes it a challenge to food safety (Park and Stoloff, 1989). Aflatoxin (AFB_1 being the most toxic) is produced on various food crops including maize, beans and groundnuts by *Aspergillus flavus* and *Aspergillus parasiticus.* Fumonisin B_1 being the most toxic, is produced primarily in maize by *Fusarium verticulliodes and Fusarium monoliforme* (Martins et al., 2001; Voss and Haschek, 2007). Environmental conditions such as high temperature, humidity, poor soil fertility, drought, insect damage, as well as the food production chains are characteristics in most parts of Africa where diets primarily consist of maize which is susceptible to fungal growth and mycotoxins production (Lewis et al., 2005).

Contamination of food products by mycotoxins can take place at any point along the food/feed chain from the field, harvest, handling, shipment and storage under a wide range of climatic conditions (Giray et al., 2007). Aflatoxin poisoning is one of the most common and most under-reported causes of toxicoses in poultry (Pattison et al., 2007). Aflatoxicosis in poultry and farm animals also causes changes in biochemical and hematological parameters, liver and kidney abnormalities, impaired immunity, and may induce mortality among animals, secondary contamination of human consumers via eggs, meat and milk (Shashidhara and Devegowda, 2003). Fumonisin (FB_1) also suppresses the immune system, causes deficiency in nutrients such as folic acid (Blom et al., 2006) and the modification of sphingomyelin metabolism (Stockmann-Juvala and Savolainen, 2008).

Research conducted in Nigeria, Tanzania and India on animal feed commodities showed mycotoxin contamination (Kehinde et al., 2014). Kajuna et al. (2013) in a study involving poultry feed in Tanzania reported that, 68% of all feed samples were contaminated with aflatoxin B_1. In Haryana, India, poultry feed samples analysed by Jindal et al. (1999) contained fumonisin B_1 contamination.

According to FAO report (2013), the rate of population growth over the past five decades has increased globally and has led to the rapid growth in the meat sector by rising demand of poultry meat, which has increased

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by threefold. The poultry industry has been a contributing area in agriculture that leads the economy with 34.5% share of the total growth domestic product in Ghana (Attuahene et al., 2012). Poisoning of poultry feed with mycotoxin could affect the total growth per domestic product of economy growth if not well investigated. Globally, maize (corn) and soybean are used in the preparation of poultry feed (Ensminger et al., 1990). Studies in Ghana have demonstrated contamination of weanimix food (a cereal-legume blend food) with aflatoxin and fumonisin (Kumi et al., 2014). Since a large number of poultry feed are prepared from these cereals, there is the possibility of mycotoxins contamination of poultry feed from Ghana. However, studies regarding aflatoxin and fumonisin levels in poultry feed in the animal industry are readily not available in Ghana.

Thus, the aim of the study was to investigate the levels of total aflatoxin (B1, B2, G1 and G2) and fumonisin B1 in poultry feed produced in Ghana.

MATERIALS AND METHODS

Study sites

Four major poultry producing regions in Ghana, which include Brong Ahafo, Ashanti, Accra and Western regions of Ghana, were selected for this study. The Brong Ahafo region is the largest poultry producing region which contributes to about 29.62% of poultry production followed by, 28.07%, 10.72% and 9.99% from Ashanti, Eastern and Western regions of Ghana respectively (FAO, 2014).

Sample collection

The poultry feed samples, which were made up of a blend of maize, wheat grain and wheat bran were collected from farmers who produced their own feed locally in Ghana. One hundred poultry farms were selected randomly for the study. Forty samples were collected from Accra, 20 from Ashanti, 21 from Western and 19 from Brong Ahafo regions of Ghana. Hundred grams of poultry feed samples, which were made up of layer mash, broiler starter, and grower mash were collected into sterile zip-locked bags and transported at room temperature to the Noguchi Memorial Institute for Medical Research. All laboratory work was conducted at the Noguchi Memorial Institute for Medical Research, University of Ghana.

Aflatoxin measurement

Sample preparation. About 50g of poultry feed was measured and blended with 5g sodium chloride (NaCl) and 100mls of 80% methanol in a blender at high speed for 1-2 minutes. The supernatant was collected and filtered twice using a filter paper. The filtered supernatant (10 mL) was measured into a clean vessel, diluted with 40mL of distilled water. Diluted extract was filtered twice through glass microfibre filter into a clean vessel.

Affinity chromatography. The diluted extract, 5 mL was passed through Afla Test affinity column at a rate of about 1- 2 drops/second. The column was washed with 10 mL of distilled water. The extracted aflatoxin in the poultry feed was eluted with1.0 mL HPLC grade methanol into a clean test tube.

Total aflatoxin quantification. Total aflatoxin quantification was done using a calibrated high-performance liquid chromatography with fluorescence detection of wavelength 365nm excitation and 425nm emission.

Fumonisin measurement

Sample extraction. Poultry feed sample (50g) and 5g sodium chloride (NaCl) were measured and blended with 100 mL of 80% methanol in a blender for 1-2minutes. The supernatant was collected and filtered twice using a filter paper and diluted 4-fold with 0.1% Tween-20.

Affinity chromatography. Five (5 mL) of filtered diluted extract was passed through fumonitest affinity column at a rate of about 1- 2 drops/second followed by 10 mL wash with distilled water. Fumonisin in the feed was eluted by passing 1.0 mL HPLC grade of methanol through column at a rate of 1-2 drops/second while collecting the entire sample eluted in a test tube.

Fumonisin quantification. Fumonisin developer (1.0 mL) was added to the eluted sample, mixed and read at a fluorescence detection of 483nm after 240 seconds using a calibrated fluorometer (VICAM, series 4) with detection limit 0.2 ppm (2 mg/kg).

Statistical analyses

All statistical analyses were performed using Sigma Stat (2012). The data obtained from measurement of mycotoxins (total aflatoxin and fumonisin B1) from the four regions of Ghana (Accra, Ashanti, Western and Brong Ahafo) were represented by tables and figures and subjected to simple descriptive statistics, which looked at the range and the mean.

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RESULTS

Total aflatoxin (B1, B2, G1and G2) and fumonisin B_1 standard calibration curves were established with an R^2= 0.9913 and 0.9887 respectively (Figures 1 and 2) to determine the linearity of the HPLC system. Out of 100 samples collected, total aflatoxin contamination were detected in all samples in a range of 0.02-22 ppb. Three samples representing 3% of the total samples collected were detected to have total aflatoxin levels greater than 20 ppb (Table 1). Fumonisin contamination was detected in all 100 samples in the range of 0.5-4.6 ppm (Table 2). There was no statistical significant differences in occurrence of mycotoxins (total aflatoxin and fumonisin B1) from the various regions with P=0.530 and 0.612 for aflatoxin and fumonisin respectively (p significant was set to <0.05 with 95 percent confident level). Out of the 100 questionnaire analyses, 70 of the poultry feed samples collected, representing Greater Accra Region, Ashanti Region, Western Region, and Brong Ahafo Region had their feed mixed with antifungal binder to prevent fungal contamination whiles 30% did not use antifungal binders (Figure 3).

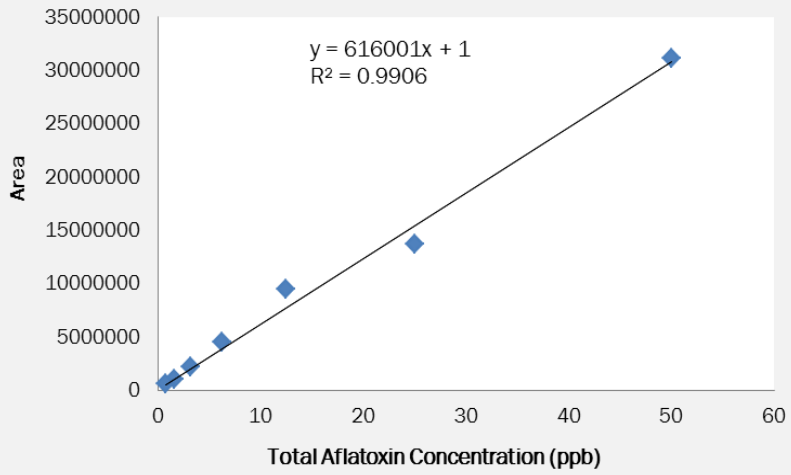

Figure 1 - Standard curve of total Aflatoxin, HPLC at excitation: 365nm, emission: 425nm

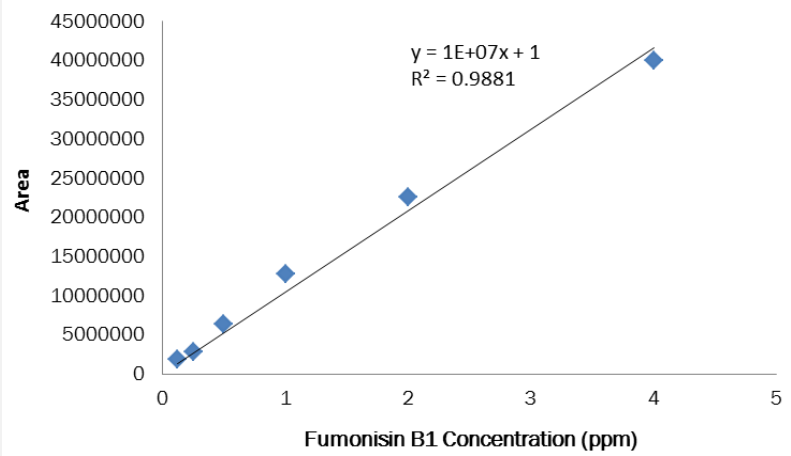

Figure 2 - Standard curve of Fumonisin B_1, at fluorescence 483nm

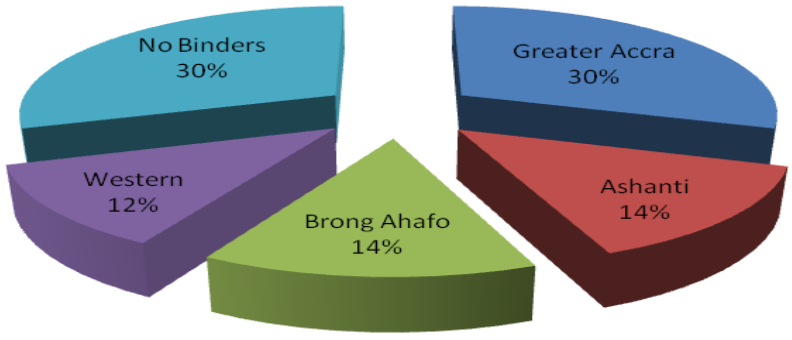

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Figure 3 - Percentage of poultry feed with antifungal binders

Table 1 - Total Aflatoxin Levels in Poultry feed Produced in Ghana

Region	No of Samples	Mean TAF (ppb)	Range TAF (ppb)	TAF > 20 ppb
Accra	40	3.0	0.1 - 21	1
Ashanti	20	2.4	0.05 - 22	0
Western	21	1.3	0.02 - 3.9	0
Brong Ahafo	19	4.7	0.8 - 22	2

n=100. TAF: Total Aflatoxin

Table 2 – Fumonisin B1 Levels in Poultry Feed Produced in Ghana

Region	Total No. of Samples	Mean FB_1 (ppm)	Range FB_1 (ppm)	No Samples > 50 ppm
Accra	40	2.7	0.8 - 3.1	0
Ashanti	20	1.5	0.3 - 4.6	0
Western	21	1.2	0.08 - 1.4	0
Brong Ahafo	19	0.3	0.5 - 1.5	0

n=100. FB_1: Fumonsin B1

DISCUSSION

The poultry industry has been a contributing area in agriculture which leads the economy with 34.5% share of the total growth domestic product (GDP) in Ghana (Attuahene et al., 2012). Occurrence of mycotoxins in poultry feed and feed ingredients are of worldwide concern since they reduce poultry performance and could be important vehicles for introducing aflatoxins residues into the human diet (Ardic et al., 2008). Consequently, many countries have regulated the maximum permissible levels of aflatoxin in food and feed products to reduce the hazard of aflatoxin poisoning but in terms of its economic considerations; these regulations vary in different countries (Stoloff et al., 1991).

Mycotoxins (total aflatoxin and fumonisin B1) were detected in all poultry feed samples analysed. The United States Food and Drug Administration, regulatory affairs and center for veterinary medicine guidelines for aflatoxin regulate the limit of aflatoxin levels in poultry feed as 20µg/kg (US FDA, 2019). According to the results of the present study, 97 feed samples representing 97% were below the permissible aflatoxin level of 20ug/kg in poultry feed. Three feed samples representing 3% recorded total aflatoxin levels above the permissible level of (Table 1). The three feed samples which were above the permissible aflatoxin levels were from samples, received from farmers who did not add fungal binders to their feed (Figure 3). Similar to the findings of the present study, another study conducted in Iran reported low aflatoxin levels ranged between 0.05 and 5.38µg/kg detected in 75 Iranian poultry feed samples (Mayahi et al., 2007). Aflatoxin B1 contamination was also found in sorghum and maize from India in the range 5-125 and 0.38-109 µg/kg respectively which constitute the major ingredients of poultry feed (Shetty and Bhatl, 1997).

The presence of mycotoxin in poultry feed could results from the feed ingredients and raw materials used in their production (Lozada, 1995). Several mycotoxin binders have been developed that prevent harmful effects of mycotoxins on animals consuming contaminated feed. In a study conducted by Kolosova and Stroka (2012), commercial aflatoxin binders containing hydrated sodium calcium aluminosilicate was used as the main component in animal feed and showed a significant effect of 41% reduction on the amount of aflatoxin analysed. Seventy percent of the poultry feed obtained from farmers in the present study included fungal binders to their feed (Figure 3). The low levels of aflatoxin recorded in the present study could be due to the addition of fungal binders to poultry feed.

Maize and maize-based products were found to have the highest occurrence and mean concentrations of fumonisin B1 than any other cereal or cereal-based product in an evaluation by the Joint FAO/WHO Expert Committee meeting on Food Additives in 2016 in Rome, higher mean concentrations of fumonisin B1 was reported in products from Africa, Central and South America and some countries in the Western Pacific Region. In a study by Shetty and Bhat (1997), fumonisin B1 contamination in poultry feed were determined in the range of 0.02 to 0.26mg/kg. The present study reported fumonisin contamination in poultry feed in the range of 0.8-4.6 mg/kg (Table 2) which was lower than the permissible limit of 50 mg/kg set by the United States Food and Drug Administration (US FDA, 2001). The present study concurs to the report by Shetty and Bhat (1997) who reported similar findings. The low levels of fumonisin in poultry feed detected in the present study could be due to the fact

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that, majority of farmers who treated their feed with fungal binders (Figure 3). Majority of the poultry feed samples analysed had low levels of total aflatoxin and fumonisin B1.

CONCLUSION AND RECOMMENDATIONS

In accordance with the findings of the present study, it can be concluded that, most of the poultry feed samples collected and analyzed were within the permissible limit of total aflatoxin and fumonisin B1 consumption by poultry birds. Fungal binders were found to be helpful in reducing the amount of aflatoxin and fumonisin levels in poultry feed. Poultry farmers are encouraged to include antifungal binders to their feed before consumption by poultry birds. The present study for the first time in Ghana, probably has demonstrate the levels and the need to monitor poultry feed for mycotoxin contamination. However not withstanding farmers need to be educated on the dangers of mycotoxin poisoning to their birds.

DECLARATIONS

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Authors' Contribution

J.Kumi participated in the proposal design of the study, prepared the manuscript in writing and performed most of the laboratory analysis. Mr. Kofi Aaron Agyei-Henaku worked on reviewing and editing the proposal, recruitment of farmers into the study as well as contributed to laboratory analysis. Mark Ofosuhene contributed to the final proposal design. All authors have read the manuscript before submitting for publication.

Availability of data and materials

Data will be made available on request from the primary author.

Consent to publish

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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POULTRY FEED RESOURCES AND CHEMICAL COMPOSITION OF CROP CONTENT OF SCAVENGING INDIGENOUS CHICKEN

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ABSTRACT: The study was conducted in Genji district of West Wollega Zone with the objectives of characterization of scavenging poultry feed resource base (SFRB) and evaluation of composition of crop content of scavenging indigenous chicken. A total of 60 sampled grower chickens (50% female and 50% male) at an age of 4-6 month, were purchased fromrural farmers and slaughtered during early dry season to study the physical characteristics and chemical composition of the crop content. About 50.7, 23.85, 12, 8.4 and 5.2% of the crop contents of experimental chickens were cereal grains, house-hold leftover/kitchen waste, animal proteins (insects/worms), plant/leaves, and none feed materials respectively. There was variation in composition with altitude and sex of birds slaughtered. The mean weight of the crop content obtained from the cockerels (25 g/day) was significantly lower ($P<0.05$) than that of the pullets (34 g/day, but there were no significant difference between altitude in mean weight of crop content of the experimental birds slaughtered. According to the result of laboratory analysis, the dry matter, ether extract, ash, crude protein, crude fiber, nitrogen free extract and calculated metabolizable energy content of the crop content were 89.37, 2.48, 14.82, 10.88, 9.35, 62.61% and 2552.3 kcal/kg), respectively. The percent composition of dry matter, ash, crude fiber, and calcium were significantly ($P<0.05$) higher in the crop content of pullets than in the crop content of cockerels, while crude fiber and crude protein level of the crop contents of the chickens of the mid altitude were significantly higher ($P<0.05$) than that of the crop content of chickens of the low altitude. The study showed that the nutrient contents of Scavengable feed resources were below the bird's requirements for optimum productivity. In conclusion, Poultry keepers must provide sufficient supplementation to their birds rather than simply throwing leftovers away to the birds.

Keywords: Chemical Composition, Crop Content, Indigenous Chicken, Scavengable Feed Resources.

INTRODUCTION

The Ethiopian poultry industry is dominated by traditional management system in which the birds are left to scavenge for their nutrients with little or no supplementation and separate poultry housing. The scavenging village poultry is of enormous socio-economic significance in Ethiopia, in terms of contribution to human nutrition and family income (Muchadeyi et al., 2007), indicating that the poultry sub-sector has the potential to provide relatively affordable animal protein.The Ethiopian chicken population is estimated to be 59.5 million of which 90.85% are indigenous, 4.76% hybrid and 4.39% are exotic (CSA, 2017). The Ethiopian indigenous chickens show a large variation in body conformation, plumage colour, comb type and productivity (Hassan, 2007). According to CSA (2017) about 37.93%, 16.04%, and 46.03% of the national poultry population are chicks up to 8 weeks, growers aged between 9 and 20 weeks and adult birds of more than 20 weeks of age respectively. About 36.21 % of the total national standing chicken population is hens of which about 2.74% are non-layers. The four major Regional States in terms of land area and human population (Oromia, Amhara, SNNP, and Tigray) collectively account for about 96.32% of the total national poultry population. Oromia region own about 34.4% of the total national chicken population and contributes 36% of the total annual national egg and poultry meat production. The region's rural areas constitute about 97.1% of the total regional chicken population while the urban areas constitute 2.9%. West Wollega Zone is accountable for about 6.65 % of the total regional chicken population (CSA, 2017).

However, the economic contribution of the Ethiopian poultry sub-sector is not proportional to the huge chicken population of the country, due to the presence of many productions, reproduction and infrastructural constraints (Aberra, 2000; Hassen, 2007). The major poultry production constraints are that of availability, quality and cost of feed ingredients. There is no planned feeding of chickens under traditional village production in Ethiopia and scavenging is almost the only source of diet. The scavenging feed resource base for local birds is inadequate and variable depending on

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season (Yami and Dessie, 1997). There may be deliberate supplementary grain feeding during food crop ripening and harvesting Period. The quantities of supplementation depend on seasonal variation. Scavenging chickens are vulnerable to predation as they need to leave the family dwelling to scavenge for feed. Scavenging for feed away from the family dwelling also results in birds coming into contact with larger numbers of birds from other flocks, facilitating the spread of infection. Newcastle disease is usually cited as the most widespread, particularly during the rainy season.

The Scavenging Feed Resource Base (SFRB) used under the traditional production system, consists of household wastes and edible materials found in the immediate environment, together with a small amount of grain supplements provided by the household (Mehari, 2016). Bekele (2016) indicated that shortage of feed restricted the potential productivity of scavenging local birds to 40-60 eggs/hen/year. According to Hayat et al. (2016), the nutrient content of the scavenging feed resource base is below the requirements of the scavenging local chickens and the available scavenging feed resource is inadequate in quantity and deficient in all the nutrients required.

Unfortunately, however, the amount of feed available for scavenging in relation to the carrying capacity of the land areas and flock dynamics across the different seasons and agro-ecologies is still not adequately quantified. Scavenging poultry feed resources, its challenges and coping mechanisms are significant gaps that need to be assessed for the purpose of intervention. Feed is one of the cornerstone challenges of poultry production. The available scavenging feed resource base need to be identified aimed at the rational utilization of locally available feed resources. This being the case, the objective of this research was characterization of scavenging poultry feed resource base and chemical composition of crop content.

MATERIALS AND METHODS

Ethical approval

The scientific and ethics committee of the College of Agriculture and Veterinary Medicine, Jimma University approved the study protocol.

Description of the Study Area

This study was conducted in Genji district (woreda) of Western Wollega Zone of Oromia Regional State, located at 544 km west of Addis Ababa. Geographically, Genji district is located at southwest of West Wollega Zone between 8^{0}57,30" and 9^{0}7,30" North latitude and 35^{0}30,0" and 35^{0}45,0" East longitude. The woreda was stratified in to two agro ecological zones (mid altitude and low altitude), ranging between 1420 and 2500 m.a.s.l. The minimum and maximum annual temperature of the woreda varies from 16 to 25°C, respectively. The study area received annual rainfall ranging between 1225 and 2000 millimeter. The soil type found in the study area is clay (80%) and sandy soil (20%).

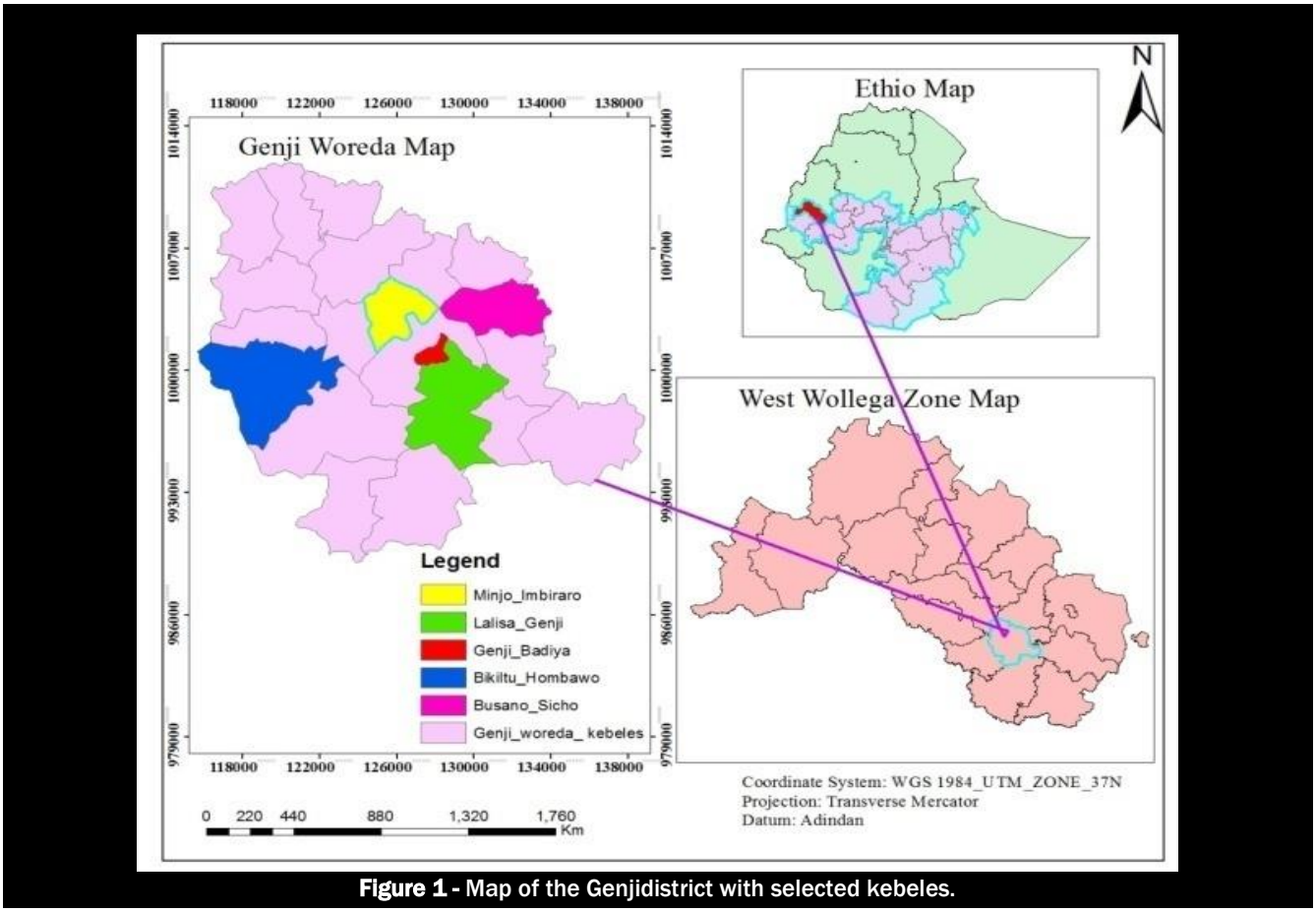

Figure 1 - Map of the Genjidistrict with selected kebeles.

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Study of Crop Contents

Thirty households were selected based on ownership of scavenging chicken and willingness to further participate on crop content experiment. A contractual agreement was arranged with a total of 30 households (6 farmers from each Kebele) for the purpose of purchasing experimental chickens. A total of 60 grower chickens (30 pullets and 30 cockerels) between an age of 4-6 months (six from each Kebele) were purchased on the basis of physical appearance and information provided by the participating households. The chickens purchased were picked up from the households flock between 5.00 and 6.00 pm (local time) considered being the last hours of scavenging. The chickens were directly transported to the Genji town slaughter house in cages and slaughtered between 6:00 and 7:00 pm. The birds were individually weighed, slaughtered and allowed to bleed for five minutes. This was followed by socking in boiled water, feather plucking and evisceration. Crops were carefully removed from each of the slaughtered bird and weighed using sensitive electronic balance. Crop material was visually examined and separated in to different categories. Finally the crop content was air dried for one day and transported to Addis Ababa'JIJE' Analytical Testing Service laboratory for chemical analysis.

Laboratory Chemical Analysis

The crop content was oven dried at 65^{0}c to a constant weight and was grinded to pass through 1 mm screen. The grinded materials were stored in tight plastic bags until required for laboratory chemical analysis. The materials were analyzed for proximate components i.e. the Dry Matter (DM) content was determined according to the standards of the Association of Official Analytical Chemists (AOAC, 2000) official methods the crop content per sample was heated in an Oven dry at 130^{o}c for 3 hours. Crude protein was determined using nitrogen to protein conversion factor of 6.25to convert total nitrogen to CP. The crude fiber (CF) was determined according to AOAC (2000) using Ceramic Fiber Filter method. Ether Extracts was determined according to AOAC (2000) Official method using Sohxlet apparatus.

Ash content was determined according to AOAC (2000) Official direct method using incinerating the sample at 550 ^{0}C for 16 hours. Calcium was determined according to AOAC (2000) with the use of EDTA Titration. Phosphorus was determined according to AOAC (2000) with the use of Vanadomolybdo phosphoric acid. Nitrogen Free Extract (NFE) was calculated by difference as: 100 – (CP+CF+EE+Ash). The Metabolizable Energy content of the materials was estimated using the formula adopted by Wiseman, (1987), with the assumption that TME is 8% higher than ME (Rashid et al., 2005).

TME (Kcal/ kg^{-1} DM) = (3951 + 54.4EE - 88.7CF - 40.8Ash).

Where: EE= % composition of ether extract on DM basis. CF= % composition of crude fiber on DM basis. Ash= % composition of total ash on DM basis. The conversion factor of 238.85 kilo calorie (Kcal) equivalents to 1 Mega joule (MJ) was used to convert Kcal to MJ.

Statistical Data Analysis

Data collected from crop content of experimental chicken and laboratory chemical analysis were normally distributed and analyzed using the General Linear Models (GLM) procedure using SAS, 2009 (Version 9.3) (Littell et al., 2002). The 5% significant level should be considered based on the following model.

Y = μ +si +aj + (sa)ij + Eij

Yij= an observation for a given variable
μ = overall mean.
si = effect of the ith sex of the bird (i= 1 cockerel , 2 pullets)
aj=effect of the jth altitude of the study area (j = 1 low altitude, 2 Mid altitude)
(as) ij = effect of interaction between sex and altitude of the study area..
Eij = residual random error.

RESULTS AND DISCUSSION

Weights of live bird and crop content

The results of the quantity and crop content of the experimental birds are given in Table 2. The results showed that the mean live weight of the cockerels and pullets slaughtered for the determination of crop content was 1.4 and 1.23 kg/ bird respectively. The result showed that the mean live weight of the experimental sampled pullet were significantly lower ($P<0.05$) than the mean live weight of the cockerels. The result of the current study was in line with that of Hayat et al. (2016) who reported that the mean live weight of indigenous pullets and cockerels in SekaChokorsa was 1.12 and 1.4 kg/head, respectively. There was significant difference ($P<0.05$) between cockerels and pullets in mean body weight at slaughtering while there was no significant difference ($P>0.05$) between either the experimental cockerels or pullets obtained from low land and midland agro ecology in mean body weight at slaughter. The results obtained showed that, the mean total crop content obtained from pullets and cockerels was 34 and 25g, respectively. The results of the current study was in agreement with that of Mekonnen et al. (2010) who reported that hens have better scavenging capacity probably to meet the nutrient requirement for egg production. According to McBride et al. (1999), the male would be most of the time on guard and show an alert position while females keep on scavenging. The males call to the hens when they find edible items to share through the performance of "tidbitting" displays by picking up and dropping the food repeatedly and offering it to the hen. During the breeding season, males become very territorial and guard fixed areas and dominant males patrol the boundaries of their territory.

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Physical characteristics of the crop content

The scavenging feed resources of the study area as measured by the physical visual appraisal of the crop content of slaughtered chicken comprised of household scraps, animal protein sources (worms, small snails, grass hoppers, ants termites), grains (maize, sorghum, millet, teff, barley), green materials (leafs and grass) and other inedible materials. The results of the visual appraisal indicated that (in order of importance), the major components of the crop contents were cereal grains, household scraps, insects and worms, plants and inedible materials. Significant interactions between altitude and sex of birds were observed with regard to the crop weight, grain, insects/worms, plant material and other content.

The fresh crop contents obtained from the slaughter chickens considerably varied based on altitude and sex of the slaughtered chickens (Table 1). However, there was significant difference (P<0.05) between altitudes in the cereal grain proportion of the crop content of the experimental chickens. Cereal grains represent the largest proportion of the scavengeable feed resources of both lowland and mid altitude agro-ecologies studied. The scavenging feed resource of both the lowland and mid altitude ecologies of the study area, as measured by crop content of the slaughtered chickens comprised of cereal grains, refused household, green materials, insects/worms and other materials, all of which showed some sort of variation between individual birds.

As shown in Table 1, there was no significant difference between the pullets and cockerels in mean daily insect and worm and green materials (P>0.05) proportion of the crop contents. Cereal grains comprised of about 53.3 and 48.1% of the total crop content of the slaughtered pullets and cockerels respectively. Maize, sorghum and millets were the cereal grains frequently encountered during the crop content analysis both in lowland and midland. The relatively higher proportion of cereal grains recorded from the crop content of the slaughtered chickens could be attributed to the harvest time of grains which takes place at early dry period (October– December) in the study area. The proportion of cereal grains obtained from crop content of pullets (53.3%) was significantly higher (P<0.05) than the proportion of cereal grains obtained from the crop content of cockerels (48.1%). Thus the results obtained indicated that the mean daily cereal grain and total feed material intake of the pullets was significantly (P<0.05) higher than that of the cockerels. The relatively higher mean daily grain and total feed material intake of the pullets seems to be due to the fact that females require more nutrients than males to meet their production and reproduction performance.

Table 1 - Effect of altitude and sex of birds on the crop content of experimental chickens (Mean±SE)

Altitude × Sex of birds	Composition (% fresh bases)						
	Body wt (kg)	Crop wt (gm)	Cereal grains (%)	Household Refused (%)	Insect/worm (%)	Plants (%)	Others (%)
Altitude							
Lowland (30)	1.34±0.02	28±0.3	48.1±1.18^{b}	23.43±0.89	9.88±0.38^{b}	7.37±0.28^{b}	5.86±0.38^{a}
Midland (30)	1.35±0.02	31±0.3	52.97±1.18^{a}	24.33±0.89	11.22±0.38^{a}	8.83±0.28^{a}	4.28±0.38^{b}
P-Value	0.654	0.701	0.005	0.476	0.017	0.001	0.006
Sex of birds							
Cockerels (30)	1.41±0.02^{a}	25±0.3^{b}	48.1±1.18^{b}	23.1±0.89	10.98±0.38	7.93±0.28	4.94±0.38
Pullets (30)	1.23±0.02^{b}	34±0.3^{a}	53.3±1.18^{a}	24.7±0.89	10.11±38	8.26±0.28	5.2±0.38
P-Value	0.001	0.001	0.001	0.203	0.1097	0.411	0.642
Altitude × sex of birds							
L × C	1.42±0.02^{a}	22±0.4^{b}	48.52±1.67^{ab}	22.67±1.26	10.24±0.54^{ab}	6.87±0.4^{b}	5.89±0.55^{a}
L × P	1.39±0.02^{a}	33±0.4^{b}	47.67±1.67^{b}	23.47±1.26	9.51±0.54^{b}	7.87±0.4^{ab}	4.00±0.55^{b}
M × C	1.28±0.02^{b}	27±0.4^{a}	53.37±1.67^{a}	24.19±1.26	11.73±0.54^{a}	9.00±0.4^{a}	5.83±0.55^{a}
M × P	1.28±0.02^{b}	34±0.4^{a}	52.57±1.67^{ab}	25.2±1.26	10.7±0.54^{ab}	8.67±0.4^{a}	4.57±0.55^{ab}
P-Value	0.001	0.001	0.001	0.530	0.001	0.001	0.001

^{a,b}Least square means with different superscript with in the same column are significantly different (P<0.05), SE=Standard Error, Alt×Sex=interaction between Altitude and Sex of bird, wt= weight, Kg=kilogram, gm=gram, %=percent, 30=number of slaughtered chickens, L×C=lowland with Cockerel, L×P=Lowland with Pullet, M×C=Midland with Cockerel, M×P=Midland with Pullet

The results of this study was in agreement with that of Hayat et al. (2016) who reported that cereal grains comprised the highest proportion of the crop content of the experimental chickens, in SekaChokorsa district of South Western Ethiopia. Furthermore this result was similar with that of Tadelle and Ogle (2000) who reported that seeds comprised the largest proportion of the feed materials present in the crop, followed by plant material, worms, insects and un-identified materials, respectively from study conducted in central high lands of Ethiopia. As shown in Table 1, there was no significant difference between lowland and mid altitude in mean total crop content and in the proportion of household refusal obtained from the slaughtered chickens (P>0.05). The household refusal obtained from both altitudes mainly consisted of kitchen leftovers (porridge and its waste products, refused injera, bread, potatoes and onion pills), maize, and other grain by-products generated during the traditional household food preparation.

The results obtained showed that the mean daily grain intake recorded in the low land (48.1 %) was significantly lower (P<0.05) than that recorded in the mid altitude (52.97%). Similarly, the proportion of insect and worms and green

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plant materials obtained from crop content of slaughtered chicken of midland was significantly higher (P<0.05) than that of the crop content of slaughtered chickens of the low altitude, attributed to the difference in the availability of protein source feed and green forages in the area In contrary to the result of this study, Tadelle (1996) reported that there is no difference in the availability of insects/worms with the midland and low land altitude, from crop contents of experimental chicken studied in central highland of Ethiopia. The proportion of animal protein source in the crop content of the slaughtered chickens was lower for the lowland. The inedible organic materials found in the crop of the slaughtered chickens comprised of soil, sand (grits), charcoals and others. The proportion of these materials was significantly higher (P<0.05) in the crop content of lowland chickens. But, there was no significant difference (P>0.05) in the proportion of inedible organic materials between pullets and cockerels.

Chemical composition of crop contents

The chemical composition of crop contents of the experimental chickens is shown in Table 2. There was no significant difference (P>0.05) between the crop content of slaughtered chickens of the lowland and mid altitude in percent composition of dry matter, total ash, crude fiber, metabolizable energy, calcium and phosphorus. The percentage composition of ether extract and nitrogen free extract of the crop content of the slaughtered chickens of the lowland was significantly higher (P<0.05) than that of the crop content of the slaughtered chickens of the mid altitude. On the contrary, the percentage composition of crude protein of the crop content of slaughtered chickens of the mid altitude was significantly higher (P<0.05) than that of the crop content of slaughtered chickens of the lowland altitude. Significant interactions between altitude and sex of bird were observed with regard to the DM, EE, CP, CF and NFE level of composition (Table 2). The relatively higher percentage of the crude protein content of the crop content of slaughtered chicken of the mid altitude could be attributed to the better availability of protein rich scavenging feed resources.

The dry matter contents of experimental pullets and cockerels were 89.86% and 89.38% , respectively and shows no significant difference between each other's (P>0.05). With the exception of the percentage composition of dry matter, there was no significant difference in the percentage composition of all the other nutrients between the crop content of the slaughtered pullets and cockerels. The mean percentage composition of dry matter and total ash of the crop contents of slaughtered pullets was higher than that of slaughtered cockerels. The relatively higher percentage of dry matter and total ash recorded from the crop content of pullets could be attributed to the high proportion of grains in their crop contents. It was also reported that the higher proportion of grains in the crop content of pullets might be a reflection of the preferential treatment given to the adult birds in grain supplementation by the local people. They believe that since the layers lay eggs or rear the chicks, they should have more feed. This result is lower than that of Mekonnen et al. (2010) who reported that the higher dry matter (91.1- 92.5%) composition of the crop content might be due to conducting the study in harvesting season. On the other side the result of the current study was contrary to that of Ncobela (2015) who reported that the dry matter concentration of crop content was higher in the hot dry season in cocks than in hens from the experimental chicken conducted in South Africa. The crude protein level of crop content of the experimental birds significantly (P<0.05) varied with altitude but there was no significant difference (P>0.05) in crude protein between the crop content of males and females (Table 2). The mean crude protein level of the crop contents of experimental birds of the mid altitude and low altitude was 12.17 and 9.47 % of the dry matter respectively.

The mean CP contents of the crop content of the study area was 10.88%, indicating that the result of the current study was lower than that of Momoh et al. (2010), who reported crude protein of 12.77% from crop content during early dry season, but was in agreement with that of 10.94% the value of which was recorded from the crop content studied during late dry season in Nigeria. The percent composition of CP obtained from the current study was higher than that of Hayat et al. (2016) who reported CP of 9.76% from crop contents of indigenous scavenging chicken of SekaChokorsa. The results of this study showed that growing chicken tended to consume feed with a higher crude protein content. According to the results of this study, the crude protein content of the crop content of the experimental birds was below the requirement (160 g Kg DM^{-1}) of local laying hens. Kinghori et al. (2003) reported that the CP requirement of indigenous chickens at 14 - 21 weeks of age is 160 g kg^{-1}. Based on the results of the current study, the total CP content of the crop content of laying hen was calculated to be 108.2 g $kg^{-1}$the value of which was lower than the requirement of laying hen. According to NRC (1994), the recommended levels of CP in diets of egg type growers range between 150 and 200 g/kg of DM. The crude protein level of the crop content obtained from the current study was lower in low altitude than in mid altitude. The low CP content of the crop contents of experimental birds from the low altitude could be attributed to the poor vegetation cover and soil fertility and relatively low proportion of seeds in the crops of the slaughtered birds.

There was no significant difference (P>0.05) between altitudes and sex of birds in the percent composition of crude fiber of the crop contents of experimental birds. Whereas, there the significant difference (P<0.05) on the interaction effect of altitude with sex of birds. On the contrary, the percentage composition of CF obtained from the crop content of pullets (9.56%) was higher than that of the cockerels (9.14%). The overall mean percent composition of CF of the crop content obtained in this study was 9.35%. This result was in line with that of Momoh et al. (2010), who reported CF content of 9.95 and 8.91% from crop contents of the Nigerian indigenous chickens during early and late dry season respectively.

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Table 2 - Effect of altitude and sex of birds on chemical composition of crop contents (Mean±SE)

Altitude/Sex of bird	Chemical Composition (% of dry weight)								
	DM	Ash	EE	CP	CF	NFE	ME (Kcal)	Ca	P
Altitude									
Lowland	89.15±0.14	14.5±0.3	2.92±0.16^{a}	9.47±0.37^{b}	9.38±0.24	63.69±0.65^{a}	2584.7±23.2	1.15±0.06	0.83±0.12
Midland	89.1±0.14	15.1±0.3	2.05±0.16^{b}	12.17±0.37^{a}	9.32±0.24	61.54±0.65^{b}	2519.9±23.2	1.01±0.06	0.65±0.12
P-Value	0.803	0.222	0.006	0.001	0.852	0.048	0.084	0.136	0.334
Sex of birds									
Cockerel	88.86±0.14^{b}	15.02±0.3	2.61±0.16	11.05±0.37	9.14±0.24	62.35±0.65	2569.1±23.2	0.92±0.06^{b}	0.68±0.12
Pullet	89.38±0.14^{a}	14.62±0.3	2.36±0.16	10.59±0.37	9.56±0.24	62.87±0.65	2535.6±23.2	1.24±0.06^{a}	0.79±0.12
P-Value	0.031	0.372	0.321	0.404	0.261	0.584	0.337	0.005	0.532
Altitude*sex of birds									
L×C	89.15±0.2^{ab}	14.71±0.42	3.22±0.23^{a}	9.5±0.53^{b}	9.6±0.34^{ab}	62.9±0.92^{ab}	2574.8±32.8	0.94±0.15	1.08±0.17
L×P	89.15±0.2^{ab}	14.38±0.42	2.62±0.23^{ab}	9.44±0.53^{b}	9.17±0.34^{ab}	64.4±0.92^{a}	2594.6±32.8	1.26±0.15	1.24±0.17
M×C	88.57±0.2^{b}	15.34±0.42	1.99±0.23^{b}	12.6±0.53^{a}	8.68±0.34^{b}	61.72±0.92^{ab}	2578.9±32.8	0.85±0.15	1.29±0.17
M×P	89.62±0.2^{a}	14.87±0.42	2.11±0.23^{b}	11.73±0.53^{a}	9.95±0.34^{a}	61.35±0.92^{b}	2476.5±32.8	1.3±0.15	1.36±0.17
P-Value	0.031	0.873	0.016	0.001	0.001	0.036	0.143	0.79	0.759

^{a,b} Least square means with different superscript within the same column are significantly different (P<0.05); SEM = Standard Error of Mean, DM= dry matter, EE= ether extract, CP= crude protein, CF= crude fiber, NFE= nitrogen free extract, ME= metabolizable energy, Ca= calcium, P= phosphorus, Alt × birds= interaction effect between altitude and sex of bird, L×C=lowland with Cockerel, L×P=Lowland with Pullet, M×C=Midland with Cockerel, M×P=Midland with Pullet,%=percent

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The CF content of crop obtained in the current study was lower than that of Hayat et al. (2016), who reported CF of 11.92 and 11.07% from crop contents of pullets and cockerels from the study conducted in SekaChokorsa. On the other side, the CF values recorded from the current study was higher than that of Mekonnen et al. (2010) and Raphulu et al. (2015), who reported CF content of 3.65 and 3.3% from crop content of egg type adult and grower chicken of Ada'a district of Oromia Region and South Africa, respectively. The CF content obtained in this studywas higher than the CF level recommended with in commercial layers rations of around 5% (Feltwell and Fox, 1978). Excessive Crude Fiber composed of cellulose, lignin and hemi-cellulose is likely to be poorly digested by mono-gastric animals (Mekonnen et al., 2010). The consumption of undesirable materials such as feathers may contribute to high levels of CF in crop contents and results in poor availability of nutrients (Sonaiya et al., 1999).

Carbohydrate is the major source of energy for poultry and most of the carbohydrate in poultry diets is provided by cereal grains. The NFE represents soluble carbohydrates and other digestible and easily utilizable non-nitrogenous substances in chicken feeding. According to the results of the current study, there was significant difference in NFE levels of crop content ($P<0.05$) between altitude and interaction effect of sex with altitude.

Mean percent composition of 62.61% of NFE value was obtained from the crop content of the experimental birds of the current study. The result of the current study was higher than that of Hayat et al. (2016), who reported NFE value of 46.2% from crop contents of the experimental chickens in SekaChokorsa Woreda. The result of the current study was also higher than that of Momoh et al. (2010), who indicated 53.62 and 56.26% of NFE from crop content of the experimental birds during early and late dry seasons of North Central Nigeria respectively. Ncobela (2015) reported 33.9 and 30.02% of NFE from crop contents of experimental chicken in South Africa. Rashid et al. (2005) and Raphulu et al. (2015) reported about 68.7 and 61.03% of NFE from crop contents of the experimental chicken in Bangladesh and Venda region of South Africa, respectively. The higher NFE value indicates higher proportion of grains in the crops during late and early dry seasons. Higher NFE content is associated with higher metabolizable energy.

The mean calculated metabolizable energy level of the crop contents of the experimental chicken of the current study was 2552.3 Kcal/kg. Tadelle and Ogle (2000) reported comparable metabolizable energy content of 2245.1–3528.1 Kcal/kg DM-1 from crop contents of the experimental chickens of central highland of Ethiopia. Higher energy content was reported during early dry season compared to the other seasons, attributed to the better availability of cereal grains which had just been harvested and given to the birds in larger amounts. The result of the current study was higher than that of Hayat et al. (2016) who reported 2023 and 2082 Kcal/kg-1 for pullets and cockerels respectively.

Momoh et al. (2010) reported metabolizable energy value of 2352 and 2598 Kcal/kg-1 from crop content of layers and grower during the early dry period in Nigeria. There is no an efficient utilization of the metabolizable energy by scavenging chickens since some energy could be lost due to their movement over a long distance to find feed. According to NRC (1994) the relative amounts of energy available vary with the amount and composition of the feedstuffs in the scavenging feed resource. Other factors, such as species, genetic makeup and age of poultry, as well as the environmental conditions also influence the utilization of dietary energy. Deficiency of energy negatively affects the production performance of poultry. If the available energy concentration of the diet is changed, birds maintain constant energy intakes by changing their feed intakes. Therefore, energy is required for chickens for supporting movement activities during scavenging.

The Ash level of the crop content of the experimental chickens was not significantly influenced ($P>0.05$) by altitude and sex of the birds. The mean ash content of the crop content observed in this study was 14.82%. This result was in agreement with that of Tadelle and Ogle (2000) who reported ash content of 1.6–15.7% from the crop content of indigenous chickens during dry season in Central Highland of Ethiopia. The result of current study was lower than that of Hayat et al. (2016), who reported ash content of 22.86and 22.15% for crop content of pullets and cockerels in SekaChokorsa.

Calcium levels of the crop contents of the experimental chicken was significantly ($P<0.05$) higher (1.24%) in pullets than in (0.92%) in the cockerels. The overall mean calcium content of experimental chicken was 1.1%. The result of the current study was lower than 1.32% reported by Rashid et al. (2004) from Pakistan. The higher calcium content in the crop content of pullets compared to that of cockerels might be attributed to a selective feeding habit of the pullets which in turn depends upon the nutritional requirement during the early phase of laying period. Calcium requirement (18 g kg DM^{-1} of laying hens is comparatively high and increases with the rate of egg production and age of the hen.

The phosphorus content of the crop content obtained in the current study was 0.68% and 0.79% for cockerels and pullets, respectively. The mean phosphorus content of the crop content of the experimental chicken in the study area was 0.74%. This result is higher than that of Tadelle and Ogle (2000), who reported 0.9 and 0.6% of Calcium and Phosphorus from crop content of indigenous chickens in the Central Highland of Ethiopia. The result of this study was in line with that of Hayat et al. (2016) who reported calcium content of 1.26 and 0.73% for pullets and cockerels, and phosphorus content of 0.66 and 0.68% from a study conducted in SekaChokorsa Woreda. The result of the current study was higher than that of Mekonnen et al. (2010) who reported 0.43-0.9% Calcium and 0.24–0.38 % phosphorus from the crop content in Ada'a district. Rashid et al. (2005) reported 0.46 and 0.34% of Phosphorus from crop content of scavenging layers and growers in Bangladesh. Poultry need Phosphorus and Calcium to build and maintain their skeletons. Phosphorus is also necessary for energy utilization at the cellular level (NRC, 1994). However, phosphorus is unavailable to the birds because it is found in the phytate form which is being excreted in to the environment (Tahir et al., 2012). The low Calcium content obtained

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from the current study indicate low availability of green forage during dry season in the study area. It is indicated that lower proportion of green forages was found in crop content of most village chickens during the harvesting season.

CONCLUSION

The present study indicated that the quantity of scavengeable feed resources in the Genji district and their chemical composition were varied with agro ecologies. The result further indicated that feed resources scavenged by local chickens were showed variation among sex of birds. During the study period the availability of cereal grains and house hold refusal /kitchen wastes were higher when compared with green plants and insects/worms observed in the crop contents of local chickens. It can also be concluded that the major nutrients such as calcium, crude protein and Metabolizable energy in crop contents appeared to be low in nutritional status of scavenging local chickens under small holder management condition except for Phosphorus. Generally the results of the current study indicated that the nutrient content of scavengeable feed resources base of Genji district is below the requirements of scavenging local chickens. This might be attributed to the lack of knowledge about the importance of supplementary feeding and very little attention given for poultry management. A wide range cereal grains available in the district to be used as supplementary source of energy. However, scavengeable feed resources alone cannot support optimal growth and egg production of local chickens. Therefore, adequate feed supplementation based on composition of the available SFR is necessary for improved productivity of the local scavenging chickens.

DECLARATIONS

Consent to publish

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Admasu Shuna (AS) conceived the study, coordinated the overall activity, and carried out the statistical analysis, drafted the manuscript. Solomon Demeke (SD) participated in drafting and reviewing the manuscript and conceived the study, coordinated the overall activity, and reviewed the manuscript. Meseret Molla (MM) participated in drafting and reviewing the manuscript. 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 up on request of the primary author

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PREVALENCE AND ECONOMIC SIGNIFICANCE OF BOVINE FASCIOLOSIS IN CATTLE SLAUGHTERED AT DEBRE-TABOR MUNICIPAL ABATTOIR

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Supporting Information

RESEARCH ARTICLE

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ABSTRACT: A cross sectional study was carried out from January, 2018 to June 2018 to determine the abattoir prevalence and economic loss associated with fasciolosis in cattle slaughter at Debretabor municipal abattoir. From the total of 350 examined cattle, 100 (28.6%) were positive for fasciolosis. Highest prevalence was observed in poor body condition cattle 26 (45.6%) followed by medium 54 (26.7%) and good body condition cattle 20 (22%), respectively. There was also significant difference in different age group. The highest 18 (40.9%) prevalence was in young cattle and the lowest 82 (26.8%) found in adult animals. Also the prevalence of bovine fasciolosis was highest in local breed 90 (32.2%) than in cross breed 7 (3.3%) with statistical significant difference (P<0.05). The total annual economic loss was estimated 60746.4 ETB. The study showed that the prevalence and money loss due to fasciolosis in cattle slaughtered at Debretabor municipal abattoir was high. Hence, immediate prevention and control of *fasciola* is needed.

Keywords: Cattle, Debretabor, Economic significance, Fasciolosis, Prevalence

INTRODUCTION

Ethiopia owns huge number of ruminants having high contribution for meat consumption and generates cash income from export of live animals, meat, edible organs and skin. In spite of the presence of huge ruminant population, Ethiopia fails to optimally exploit these resources due to a number of factors such as recurrent drought, infrastructures problem, rampant animal diseases, poor nutrition, poor husbandry practices, shortage of trained man power and lack of government policies for disease prevention and control. Cattle suffer from a variety of infection and non- infectious diseases. They may harbor several helminthes parasite (Andrews et al., 1992).

In Ethiopia both *fasciola hepatica* and *fasciola gigantica* have the greatest risk occurred in areas of extended high annual rainfall associated with high soil moisture and surplus water, with risk diminishing in areas of shorter wet season and lower temperatures. For *fasciola gigantica* regions in the high lands of Ethiopia and Kenya were identified as unsuitable due to in adequate thermal regime. Average annual mean temperatures of 23°c or above were found to correspond to areas below the 1200m elevation limit of *fasciola hepatica* in Ethiopia (Malone et al., 1998).

Several abattoir surveys conducted in various parts of Ethiopia have demonstrated the presence of fasciolosis, due to *f. Hepatica* and *f. Gigantica*, in ruminants. Some studies tried to demonstrate the economic losses associated with liver condemnation and evaluation of the economic loss due to fasciolosis differ in different parts of Ethiopia (Tolosa et al., 2007; Fufa et al., 2008; Gbratsadik et al., 2009; Nraddis et al., 2010 and Kassaye et al., 2012). The objective of the study was to determine the abattoir prevalence and economic loss associated with fasciolosis in cattle slaughter at debretabor municipal abattoir.

MATERIAL AND METHODS

Description of the study area

The study was conducted in Debretabor Municipal abattoir from January, 2018 to June 2018. Debretabor is found in south Gondar zone, Amhara regional state, Ethiopia. It is located at 670 km North of Addis Ababa. The minimum and maximum annual rain fall and daily temperature ranges between 1000 to 1500 mm and 20 to 25°C, respectively (SGAO, 2012).

Study design

Active abattoir survey was conducted based on cross sectional study during routine meat inspection on randomly selected cattle slaughtered at Debretabor municipal abattoir from January, 2018 to June 2018.

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Sampling technique

A cross sectional study was carried to determine the prevalence of bovine *fasciolosis*. Random sampling technique was use to collect all the necessary data from abattoir survey of the study animals.

Study methodology

The liver of each study animal was carefully examined for presence of lesions suggestive of Fasciola infection externally and sliced for confirmation. Liver flukes were recovered for differential count by incising the infected liver into fine, approximately 1 cm slices with a sharp knife. Each mature fluke was identified to species level according to its shape and size. Investigation and identification of Fasciola was done according to their distinct morphological characteristics following the standard guidelines given by Urquhart et al. (1996).

Sample size determination

The sample size was calculated according to Thrusfield (2005) by considering 27.7% expected prevalence from previous study and 5% desired absolute precision at 95 % confidence level using the following formula.

$$N = \frac{((1.96)^2(P_{exp})(1-P_{exp})}{d^2}$$

N = Total number of sample size represented a considerable economic and public health; P_{exp} = Expected prevalence; d = Absolute precision. Therefore, the sample was about 308. But to increase the accuracy of the study the determined sample size was 350.

Study animals

The study animals were cross breed and indigenous zebu cattle brought from various localities to Debretabor municipal abattoir for slaughtering.

Economic loss assessments

Generally, all infected livers with fasciolosis were considered to be unfit for human consumption and if any liver was infected by Fasciola at the Debretabor municipal abattoir, it was totally condemned. Economic losses were calculated based on condemned livers due to fasciolosis. In the study abattoir, the average annual cattle slaughtered rate was estimated to be 3540, while mean retail price of bovine liver in Debretabor town was 60 ETB. The prevalence of bovine fasciolosis in Debretabor municipal abattoir was estimated as 28.6%. The estimated annual loss from organ condemnation is calculated according to mathematical computation using the formula set by Ogunrinade and Adegoke (1982):

ALC = CSR × LC × P; Where ALC = Annual loss from liver condemnation, CSR = mean annual cattle slaughtered at Debretabor municipal abattoir, LC = mean cost of one liver in Debretabor town, P = prevalence of bovine *fasciolosis* at Debre tabor municipal abattoir.

Data analysis

Data obtained from postmortem findings in the abattoir uploaded into Microsoft Excel 2007 spreadsheet computer program. Then, it was analyzed by using SPSS version 16.0 for windows software and Chi-square (x^2) test is applied to compare the infection status with regard to the hypothesized risk factor like age, body condition, and breed. But comparison regarding sex was not made since all cattle brought to the abattoir were male. P-valve<0.05 was accepted as statistically significant in all cases.

RESULT AND DISCUSSION

Out of 350 male cattle that were slaughtered at Debretabor municipal abattoir 100(28.6%) animals were found infected with liver fluke. The prevalence of fasciolosis was highest in local breed 93(32.2%) and lowest in cross breed 7(3.3%) (Table 1). There was a statistically significant difference (P<0.05) in the prevalence of bovine fasciolosis in different age groups. The highest (40.9%) prevalence was in young animals and the lowest (26.8%) was found in adult animals (Table 2). There was a significant difference (P<0.05) in the prevalence of bovine fasciolosis within different body condition scores. The highest prevalence (45.6%) was found in cattle with poor body condition scores and the lowest prevalence (22 %) was found in good body conditioned animals (Table 3). The economic significance of fasciolosis was analyzed based on the information obtained during postmortem examination and interview. Annual loss due to liver condemnation = £CS×coy×Roz = 3540×60×28.6%=60746.4 Ethiopian Birr was annual lost.

Fasciolosis is widespread ruminant health problem that is regard as one of the major setback to livestock production causing huge direct economic losses in Ethiopia that were reported by many workers. The current findings 28.6% at abattoir revealed that the prevalence in debretabor municipal abattoir is lower than 46.15% recorded in Jimma municipal abattoir (Tadele and Worku, 2007) and greater than 24.32% in Mekele municipal abattoir (Berhe et al., 2009) and 14% recorded at Wolaita Soddo municipal abattoir (Fufa et al., 2009). Thus, the most reasonable condition for the presence of different prevalence from area to area through the country is due to the availability of suitable snail habitats. The result of the current study showed that age has significant effect on the prevalence of bovine fasciolosis. The prevalence was higher in young animals

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than the adult. There was a decrease in infection rate (prevalence) as age increased. This may be due to the result of acquired immunity with age which is manifested by humoral immune response and tissue reaction in bovine liver due to previous challenge. There are some additional reports confirming that the increased resistance against fasciolosis (low prevalence) with age is most likely related to the high level of tissue reaction seen in bovine liver. Liver fibrosis which impedes the passage of immature flukes acquired thickening, stenosis and calcification of bile ducts, assumed unfavorable site for adult parasites and consequently fasten their expulsion. These are in agreement with experimental study conducted by Radostits et al. (2007) which confirmed the occurrence of higher infection rate in younger animals. The results of the present study indicated that body condition of the animal has significant association with the occurrence of fasciolosis.

The prevalence was higher in poor body conditioned cattle than that of medium and good body conditioned cattle's. The prevalence of fasciolosis was higher in the animals with poor body condition because this body condition in cattle is manifested when fasciolosis reaches at its chronic stage. The present finding is by far lower than the results reported by Abdul (1992) and Daniel (1995) who reported a total economic loss of 154,188 and 215,000 ETB, respectively annually in cattle due fasciolosis at Ziway and Dire-Dawa municipal abattoir, respectively. These higher values may be due to higher number of animals slaughtered at the Dire-Data and Ziway abattoirs. The ecological conditions and the number of intermediate host found around the area may also be another factor contributing to the decrement of the economic losses.

Table 1 - Prevalence of fasciolosis in local breed

Breed	Number of examined cattle	Prevalence (%)	X^2-value	P -value
Local	289	93(32.2%)	10.58	0.001
Cross	61	7(3.3%)		
Total	350	100(28.6%)	-	-

Table 2 - Prevalence of bovine Fasciola based on age

Age	Number of Examined Cattle	Prevalence (%)	X^2 value	P -value
Adult	306	82(26.8%)	103.841	0.010
Young	44	18(40.9%)		
Total	350	100(28.6%)	-	-

Table 3 - Prevalence Of Bovine Fasciola In Association With Body Condition Score

Good	Number Of Examined Cattle	Prevalence (%)	X2 Value	P-Value
Good	91	20(22%)	9.77	0.008
Medium	202	54(26.7%)	-	-
Poor	57	26(45.6%)		
Total	350	100(28.6%)		

CONCLUSION

In conclusion the current study disclosed that bovine fasciolosis an important disease causing major economic loss in Debre-tabor municipal abattoir. Based on the finding the following recommendation would be forwarded. Strategic deworming should be applied. Society should be well informed about the disease importance. Locally available control practice should be practiced just like by planting trees having molluscides activity (Endod).

DECLARATIONS

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Authors' contributions

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

Availability of data and materials

Data will be made available up on request of the primary author

Consent to publish

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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ISOLATION AND IDENTIFICATION OF *Acetobacter sp.* FROM PINEAPPLE (*Ananas comosus* L.) AS NATA STARTER

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ABSTRACT: The purpose of this study was to isolate and identify the morphological and biochemical properties of *Acetobacter sp*. Isolation is done by growing bacteria taken from pineapple (*Ananas comosus* L.) juice on Tryptic Soy Agar (TSA) media. Identification was carried out by biochemical tests namely catalase, motility, and oxygen use tests. The study was designed per descriptive analysis by evaluating and describing the collected data. The results of the morphological experiments showed that bacterial isolates isolated from pineapple had a milky white color, round shape, small size, smooth surface, flat elevation and gram-negative type. Biochemical tests showed positive reactions in the catalase test because of break-down capability of hydrogen peroxide by the enzyme catalase, while it was negative in the motility test because bacteria form a non-motile free sphere. Bacterial isolates showed a positive reaction in testing the use of oxygen because *Acetobacter sp.* need free oxygen for growth and activity. Isolation of *Acetobacter sp.* pineapple origin has macroscopic characteristics that are milky white color, round shape, smooth surface, and flat elevation. The results of the identification of *Acetobacter sp.* pineapple origin showed a positive reaction to the catalase test, which is a gram-negative bacteria and has a round shape. Future studies are recommended to conduct a polymer chain reaction test (PCR) to identify the strain of *acetobacter sp*.

Keywords: *Acetobacter sp*., Nata, Pineapple, Starter

INTRODUCTION

Nata is an extracellular polysaccharide layer (cellulose) consisting of a thin layer of gel or fine threads made by the bacterium *Acetobacter sp*. Low-calorie nata, 2.5% fiber content, and 98% water content (Sako, 2012). Nata is robust, white and transparent, has a rubbery texture, and is floating on the surface of the liquid. The texture of the nata will tend to be difficult to bite or break easily (Budiarti, 2008). The production of nata can be influenced by the concentration of sugar used, fermentation time, nitrogen sources, and nutrient content in growth media.

Acetobacter sp. is a gram-negative bacteria that are included in the acetic acid bacteria. *Acetobacter sp*. can produce cellulose (nata) biofilms. The formation of cellulose (nata) is the result of the metabolism of *Acetobacter sp*., the process of which is controlled by the plasmid (Rezaee et al., 2005). The *Acetobacter sp.* group can be found in decaying fruits such as grapes, dates, coconuts, and pineapples and also some reveals of the production of vinegar of several types of substrates (Maal and Shafiee, 2009). The nutritional value contained in pineapple makes pineapple as a growth medium for *Acetobacter sp.* The nutritional value contained in pineapple makes pineapple as a growth medium for *Acetobacter sp*. the nutritional content needed by *Acetobacter sp* to live and thrive is sucrose, glucose, water, and fiber (Sutanto, 2012). *Acetobacter sp.* physically bacteria that are able to oxidize glucose into long chains or polymers called polysaccharides or cellulose in the form of white fibers, which form gradually from a thin layer at the beginning of fermentation to reach a thickness of about 12 mm at the end of fermentation, then referred to as nata which includes secondary metabolites. In addition to secondary metabolites, *Acetobacter sp.* also produce primary metabolite in the form of acetic acid, water and energy which is reused in at the same time its metabolism (Wu et al., 2004).

Pineapple is a fruit that is suitable for use as a food processing product (Majesty et al., 2014). According to Asif (2011) states that pineapple has a lot of content, one of which is the fiber that functions in the digestive process can reduce cholesterol in the blood and reduce the risk of diabetes to heart disease. The fiber content in pineapple is about 150 grams, equivalent to half of an orange. Pineapple 100 g contains 81.72% water; 20.87% crude fiber; 17.53% carbohydrate; 4.41% protein and 13.65% reducing sugar (Nurhayati et al., 2014). besides that in the study conducted Khusna et al. (2018) showed that pineapple fruit juice can be used as a starter in making nata.

MATERIALS AND METHODS

The procedure of researching Isolation and Identification of *Acetobacter sp.* the origin of pineapple as starter nata is done in several stages starting from the preparation of tools and ingredients, fermentation of pineapple juice and isolation of

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Acetobacter sp. from pineapple juice. The raw material applied in this study is ripe pineapple. The isolation and identification stages of *Acetobacter sp*. started by grinding 300 grams of pineapple, and then filtered. 100 ml Pineapple juice was inoculated on TSA (Tryptic Soya Agar) media to obtain isolate *Acetobacter sp*. then incubated at 30°C for 24 hours, and after that, the isolation purification was carried out by taking a loop of the colony and then scraping it on the new TSA media. Purified isolates were identified by gram staining test, catalase test, motility test, oxygen use test.

Gram Staining Test

Gram staining is done using a glass slide cleaned with alcohol and then taken bacterial isolates with an ose needle aseptically and smeared on the glass object. Bacterial isolates were then dropped into violet crystals and left for 1 minute, then washed with running water and aerated to dry. Bacterial isolates are then dropped again iniodine solution and left for 1 minute, then washed with running water and air dry. Furthermore, bacterial isolates were added with 95% alcohol for 30 seconds, then was watered and aerated to dry. Bacterial isolates were then dropped in safranin for 30 seconds and washed with running water, dried with suction paper and air-dried, then observed using a microscope (Hatmanti, 2000). Gram-positive bacteria are marked with purple which indicates that they can bind violet crystalline colors, while gram-negative bacteria are characterized by pink which indicates that these bacteria are unable to bind violet crystal colors and are only colored by safranin (a counter dye) (Fitri and Yasmin, 2011).

Catalase Test

This test is done by dripping two drops of H_2O_2 on a clean slide glass. Bacterial isolates were taken using an ose needle, then transferred to the top of the slide and stirred A positive test is characterized by the formation of oxygen bubbles which indicate that the organism concerned produces the enzyme catalase which converts hydrogen peroxide into water and oxygen (Hatmanti, 2000).

Motility Test

This test is carried out using bacterial isolates inserted into the semi-solid TSA media in a test tube using a sterile puncture needle then incubated for 24 hours at 37°C. A positive test is characterized by the growth of bacteria that spreads, then the bacteria moves (motile), and if the growth of bacteria does not spread in the form of only one line, then the bacteria do not move (non-motile) (Ismail et al., 2018).

Oxygen Usage Test

Oxygen Utilization Tests were carried out with isolates inoculated with a puncture inoculation technique on the semisolid YEPDA media. Then incubated for 48hours, and then observed the location of bacterial colonies. Bacteria are aerobic when the colonies are formed above and are anaerobic when the colonies are formed below.

RESULT AND DISCUSSION

Isolation of *Acetobacter sp.*

The results of isolation for the purification of Acetobacter sp. on pure culture TSA media grew evenly on each stroke. The results of the isolation of Acetobacter sp. presented in figure 1. After being successfully isolated, this isolate was confirmed to be morphological and biochemical. Morphological identification is carried out to determine the physical form of microorganisms, while biochemical identification is carried out to determine the chemical content in the body of microorganisms.

Identification of *Acetobacter sp.*

The results of the colony morphology and cell morphology are presented in Table 1.

Figure 1 - Pure culture of *Acetobacter sp.* on TSA media

Table 1 - The character of colonies and isolate cells from pineapple

Colony		Cell	
Character	**Result**	**Character**	**Result**
Shape	Round	Shape	Round
Color	Milky white	Size	Small
Surface	Smooth shiny	Gram type	Gram-negative
Elevation	Flat	–	–

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Morphology of the colony

The results of macroscopic observations or observations of colony morphology showed that the isolates obtained had a round colony shape with a milky white color, smooth surface, and flat elevation. This is consistent with research conducted by Laras and Priyono (2012) that *Acetobacter sp*. has the characteristics of a rounded colony, white color, and flat elves. According to Azizah et al. (2012) that particular age and growth conditions influence the shape of a colony of a bacterium. The biotic and abiotic environment also influences variations in bacterial forms that occur, growth media and temperature (minimum and maximum) (Safrida et al., 2012).

Cell Morphology

Microscopic observations or cell morphological observations were carried out by gram staining. The results obtained showed that the isolate was round, small in size, and red, which indicated that the bacteria were included in gram contrary. Gram-positive bacteria are purple because the cell walls bind violet crystals more strongly while gram-negative bacteria contain more pleated, so pores quickly enlarge, and violet crystals dissolve easily when washing alcohol (Sariet al., 2013). The results of cell morphology are presented in figure 2. Gram-negative bacteria contain lipids and fat in a higher percentage than gram-positive bacteria (Ismail et al., 2018) besides gram-negative bacteria also have thinner peptidoglycan than gram-positive bacteria (Safrida et al., 2012). Gram-negative bacteria are more dangerous pathogens than Gram-positive bacteria because the outer membrane of the cell wall can protect bacteria from the host defense system. Lipopolysaccharide compounds in outer membrane gram-negative bacteria can be toxic or toxic (Dwiyanti, 2014)

Biochemical Test Identification

The parameters used for biochemical identification include catalase test, motility test, oxygen use test. The results of biochemical identification are presented in table 2.

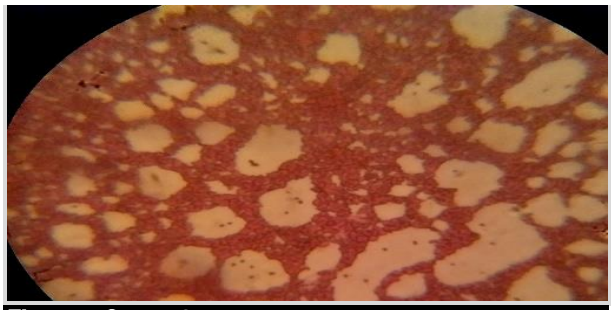

Figure 2 - Cell morphology observed on a microscope

Table 2 - Biochemical Identification Results

Identification Biochemistry	+/-
Catalase	+
Motility	-
Use of O_2	+

Catalase Test

Catalase test is important to identification the specific characteristic of isolate target. Khairiah et al, (2013) said that some Acetobacter genus has catalase enzyme as their metabolism result. The results obtained in this catalase test bacteria form gas bubbles around the colony. The formation of gas bubbles in the colony indicates that the bacteria are positive. Bacteria produce catalase enzymes that can break down H_2O_2 into H_2O and O_2. Hydrogen peroxide is formed during aerobic metabolism, so aerobic microorganisms describe the material (Fitri andYasmin, 2011). The results of the catalase test are presented in figure 4.

The mechanism of the enzyme catalase breaks down H_2O_2, which is when doing a respiration; bacteria produce various kinds of components, one of which is H_2O_2. Bacteria that can break down H_2O_2 with the catalase enzyme then immediately form a defense system from the toxic H_2O_2 that it produces itself. Positive catalase bacteria will break down H_2O_2 into H_2O and O_2 were the parameters that indicate the presence of catalase activity in the presence of oxygen bubbles (Azizah et al., 2012).

Motility Test

The motility test aims to determine the characteristics of microorganisms through indole production from tryptophane. Microorganisms can be divided into 2 properties, namely motile and non-motile The results obtained in this study are non-motile bacteria. This is consistent with what Fatmadewi (2018) did if there was no movement around the needle puncture marks on the semi-solid TSA media showed negative or non-motile results, almost all spiral bacterial cells and some stem cell bacteria were motile, whereas bacteria that were a motile non-motile round shape. The results of the motilia test are presented in figure 5.

Research conducted using TSA semi-solid media did not have any movement in the sample; it showed that the bacteria was non-motile (not moving). Immovable or non-motile bacteria can be seen by not spreading the growth of bacteria or only growing in the puncture area on semi-solid TSA media (Mergypta et al., 2014).

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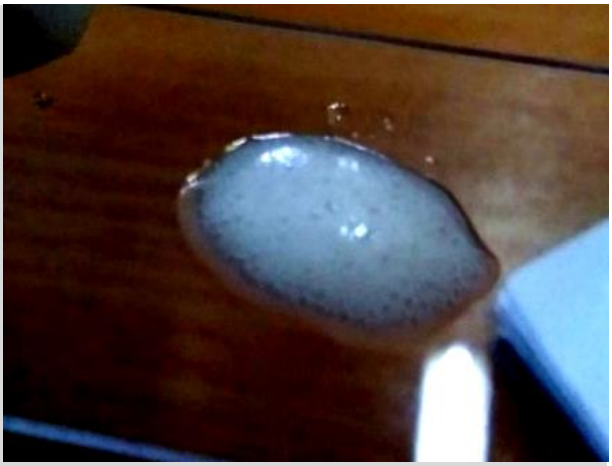

Figure 4 - Catalase test produces gas bubbles

Figure 5 - Motility test no batteries on semi-solid TSA media

Oxygen Usage Test

The results obtained in this study the colonies formed above indicate that *Acetobacter sp.* aerobic. According to Yeni et al. (2011), *Acetobacter sp.* requires oxygen for growth and activity. Fitriadi (2019) added that aerobic bacteria would be on the upper surface because they would take free oxygen from the air. According to Simanjorang et al. (2012), Acetobacter sp. has obligate aerobic properties. Obligate aerobic nature is a trait of bacteria that requires oxygen to carry out aerobic cell respiration. Aerobic bacteria in oxygen utilization are bacteria that require much oxygen as the final acceptor in biological oxidation or aerobic respiration (Fifendy, 2017).

CONCLUSION

Isolation of Acetobacter sp. pineapple origin has macroscopic characteristics that are milky white color, round shape, smooth surface, and flat elevation. The results of the identification of Acetobacter sp. pineapple origin show a positive reaction to the catalase test, which is a gram-negative bacteria and has a round shape.

DECLARATIONS

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Authors' Contribution

AK and MD designed the study and drafted the manuscript, AUP and SS performed the practical part of the experiment. MH reviewed the manuscript. All the authors approved the final manuscript.

Conflict of interests

All the authors approved and agreed to publish the manuscript.

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HISTOCHEMICAL ANALYSIS OF GASTROINTESTINAL MUCOSUBSTANCES OF FRESH WATER FISH *Mastacembelus armatus* INFECTED BY HELMINTH PARASITE *Circumonco bothrium sp.*

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Supporting Information

ABSTRACT: Present study was conducted to investigate the histochemical changes induced by *Circumonco bothrium sp.* in the intestine of freshwater fish *Mastacembelus armatus*. During present investigations the infection of *Circumonco bothrium sp.* in *Mastacembelus armatus* with various histochemical reactions showed localization of localization of carbohydrate, protein, lipid and glycogen. During histochemical study intestine infected by cestodes, the numbers of mucous cells those containing acidic or mixed glycoconjugates were significantly higher than those seen on sections from uninfected fish, which is a protective interaction of the host against parasitic infection. In the current study, a highly significant increase in the number of mucous cells was seen within the infected intestines of *Mastacembelus armatus* when compared to uninfected counterparts.

Keywords: *Circumonco bothrium sp.*, Histochemical, Intestine, *Mastacembelus armatus*

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INTRODUCTION

The gastrointestinal system is primarily involved in breaking down food for absorption in to the body. It is essentially a muscular tube lined by a mucous membrane which exhibits regional variations reflecting the changing functions of the system from mouth to anus. The Alimentary canal is an organ which is involved in various important physiological functions. It is the primary site of food digestion (absorption) and nutrient uptake.

According to Srivastava (1975) and Chandra et al. (2011), most of the species of helminths in adult stage live in the alimentary canal these parasites have detrimental effects upon fish in more ways than one. Different parts of the cell are biochemically different, they take up specific stains to varying degrees. Histochemical tests are used in an attempt to identify cell and tissue components by virtue of their specific chemical reactions. The alteration in the state of cell constituent can be studied by using histochemical techniques, these techniques helps to analyze not only the localization of carbohydrate, protein, lipid and glycogen etc. but also molecular changes at cellular level. The noteworthy contributions towards the expansion and development of histochemistry are those of Lillie (1954), McManus (1948), Pearse (1968) and Bancroft and Stevens (1992), Sonune (2014). In 2012, Ghosh and Chakrabarti observed the histochemistry of the olfactory rosette of *Cyprinus carpio*.

The present study includes the Histochemical analysis of gastrointestinal mucosubstances of fresh water fish *Mastacembelus armatus* infected by helminth parasite *Circumonco bothrium sp.*

MATERIALS AND METHODS

Preparation of slides for histochemical studies:

For histochemical analysis, small fragments from the anterior, middle and posterior parts of infected intestine were used. The infected intestine and normal were cut into small pieces and were fixed in Bouin's fluid. After 48 hours, washed several times with water, dehydrated in graded series of alcohols, cleared in Cedar wood oil and xylene, blocks were made in cavity blocks by usual method. Thick sections were cut with a rotary microtome at 4- 5 micron thick. After removing the wax by xylene, hydration was carried out, dehydrated, cleared in clove oil and xylene and mounted permanently in Canada balsam. Sections were stained with various histochemical staining methods. Best slides or sections were selected and observed under the microscope for histochemical study. Photographs were taken with digital camera Nikon Coolpix L24.

Methods used for histochemical tests were:

1. Periodic Acid- Schiff (PAS) (McManus, 1948)
2. Alpha-amilase-PAS (McManus, 1948)
3. Alcian blue pH 2.5 (Martoja and Martoja-Pierson, 1970)
4. Alcian blue pH 0.4 (Martoja and Martoja-Pierson, 1970)

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5. Congo red (Pearse, 1968)
6. Sudan black B (McManus, 1948)
7. Ferric ferricyonide (Pearse, 1968)
8. Free aldehydes (Sawhney and Randhir Singh, 2014)

All the data of results were tabulated according to color intensity into different grades ranging from + to ++++
Colour index:

1. Strong positive reaction ++++
2. Moderate reaction ++
3. Weak reaction +
4. Negative reaction -

RESULTS

During present investigations the infection of *Circumonco bothrium* sp. in *Mastacembelus armatus* with different histochemical reactions showed localization of different chemicals. The results on the detection of the different mucins in infected intestine are shown (Figures 1-8).

When the sections of intestines are stained with PAS stain, it was found that uninfected fish showed abundant PAS positive carbohydrates in the brush border of the intestine, whereas a moderate amount was seen intestinal goblet cells. The most evident change observed in parasitized fish with respect to uninfected fish was that the low goblet cells though changes in the intensity of PAS staining were not evident. Lamina propria was slightly PAS positive in both infected and uninfected fish (Figures 1 and 2). When stained with Alcian blue pH 2.5 section of infected showed moderate staining with AB staining, bluish colour with this test suggesting the presence acid mucopolysaccharide content (Figure 3). With Alcian blue pH 0.4 section of normal intestine showed sulphated mucins predominant whereas infected intestine showed few sulphated mucins (Figure 4). When stained with Congo red the infected intestine showed extensive deposition of eosinophilic amorphous material (amyloid) in the muscularis mucosa, submucosa, and muscularis propria layers of the intestine (Figure 5). With Sudan black B the normal intestine show the relatively low amount of lipid whereas infected intestine show the relatively high amount of lipid (Figure 6). When stained with Ferric ferricyonide infected intestine with *Circumonco bothrium sp.* shows moderate iron deposits in the tissue which is the indication of the disease (Figure 7). With Free aldehydes the infected intestine shows PPT whereas normal intestine do not show PPT (Figure 8).

Table 1 - Results of histochemical reactions of normal and infected intestine of freshwater fish *Mastacembelus armatus* infected with *Circumonco bothrium sp.*

Sr. No.	Stain/Method	Intensity		Inference about mucosubstances		Fig. No.
		Normal intestine	Infected intestine	Normal intestine	Infected intestine	
1	PAS	+ ++	++	Neutral mucin present	Neutral mucin present	1
2	Alpha-amilase-PAS	++	+	Mucin present	Increase mucin	2
3	Alcian – blue pH 2.5	++	+++	Acidic mucins present	Acidic mucins Increase	3
4	Alcian – blue pH 0.4	++	+	Sulphated mucins predominant	Few sulphated mucins	4
5	Congo red	++	+++	Amyloid elastic fibres few	Increase Amyloid	5
6	Sudan black B	++	+++	Less lipid	Increase lipid	6
7	Ferric ferricyonide	+	++	Less iron deposits	More iron deposits	7
8	Free aldehydes	+	++	No PPT	Dark PPT	8

DISCUSSION

A heavy mucus production has also been described from several other fish-helminth systems including those detailed by Chambers et al. (2001). The attachment organ of helminth parasites often provokes an inflammatory response within the hosts gastrointestinal tract (Dezfuli et al., 2011). Inflammation is a protective reaction in response to parasitic invasion which results stimulation of specific chemical alterations to the cellular community and tissues at the site of infection. Hur et al. (2013) although the factors that govern mucus discharge are partially defined for mammals, they are not well studied in fish. The present findings are more or less similar to the observations made by Kaur (2014). Who reported the pathological changes mainly enhanced mucus secretion in

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Channa punctatus and *Channa striatus* infected by cestode, *Senga* sp. A heavy mucus production has also been described from several other fish-helminth systems including those detailed by Benarjee and Reddy (2006).

Observation of the selected infected slides reveals that average amount of amyloid is present, which stained brownish black in colour when stained with Congo red whereas in normal intestine show the relatively low amount of amyloid. The gastrointestinal tract is typically covered by mucus, the properties of which change in different regions of the alimentary canal (Shephard, 1994). The mucus, which can be considered an aggregate secretion, is produced by mucous cells. Whereas, mucin is glycoproteins within this secretion (Theodoropoulos et al., 2001). There is a high trace of glycogen particularly in the muscularis mucosa, but in infected intestine moderate quantity of glycogen is seen (Sonune, 2014).

There is no complete agreement on the role of excessive mucus secretion which, in the intestines of fish infected with helminths, appears as protective blanket of gel or mucus. Although it has been suggested that increased mucus production in mammals may facilitate the expulsion of intestinal nematodes (Ishikawa et al., 1993), this is not the case in the current *Circumonco bothrium sp.* and *Mastacembelus armatus* situation. In the present study *Circumonco bothrium sp* ensures a secure attachment to the intestinal wall of its host even they crossed the intestine and enters the coelom. Thus it will be suggested that, the main role of mucus is to protect the underlying intestinal mucosa as a physical barrier against the mechanical and biochemical damage induced by parasites (Schroers et al., 2009).

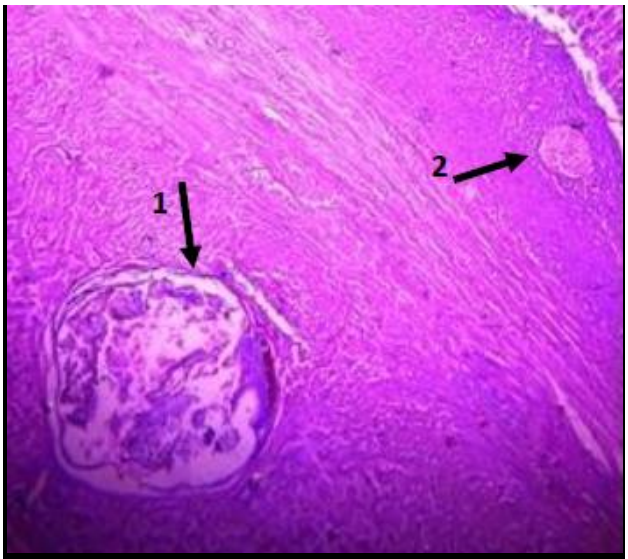

Figure 1 - Infected intestine stained with PAS (100X)

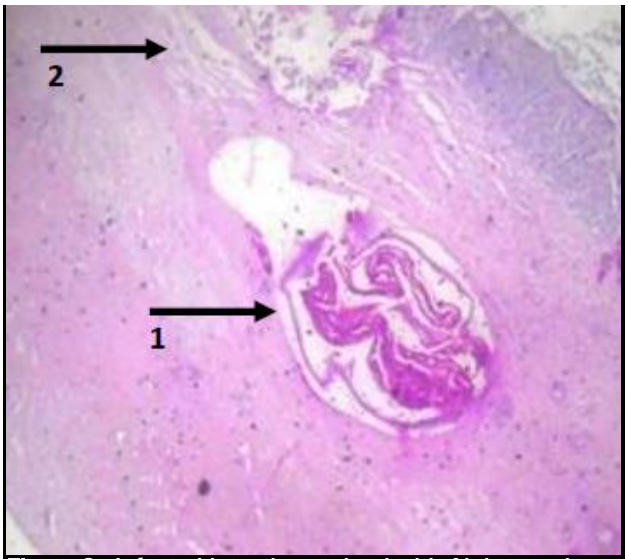

Figure 2 - Infected intestine stained with Alpha-amylase-PAS (100X)

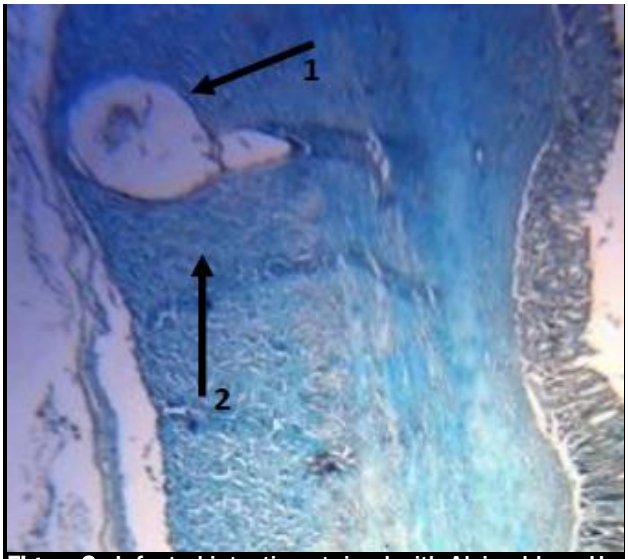

Figure 3 - Infected intestine stained with Alcian blue pH 2.5

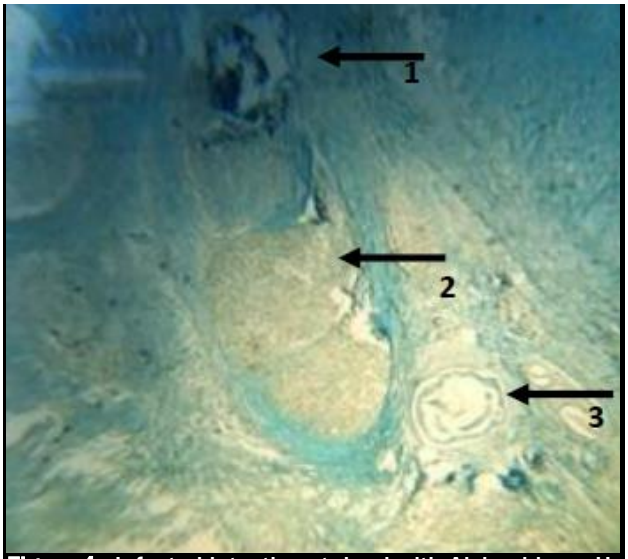

Figure 4 - Infected intestine stained with Alcian blue pH 0.4

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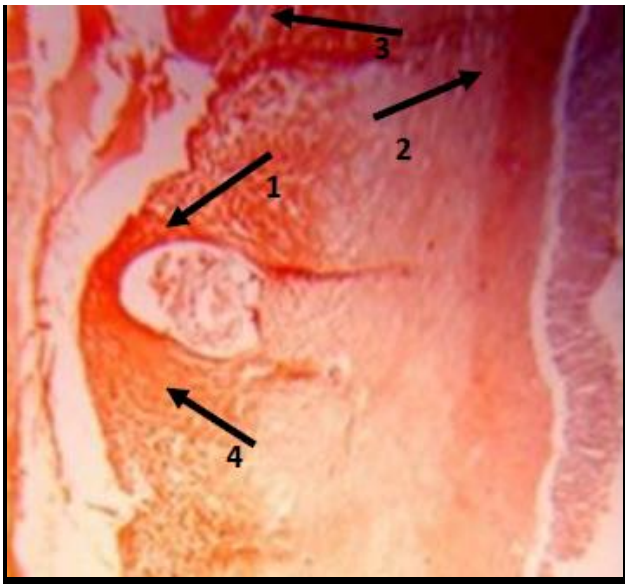

Figure 5 - Infected intestine stained with Congo red

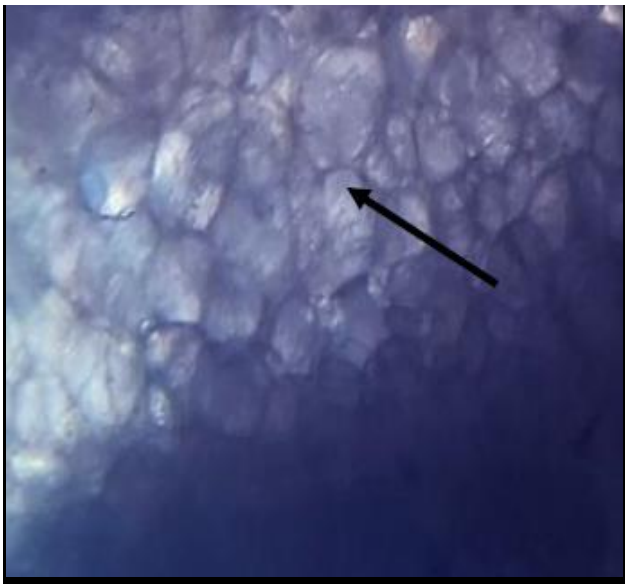

Figure 6 - Infected intestine stained with Sudan black B

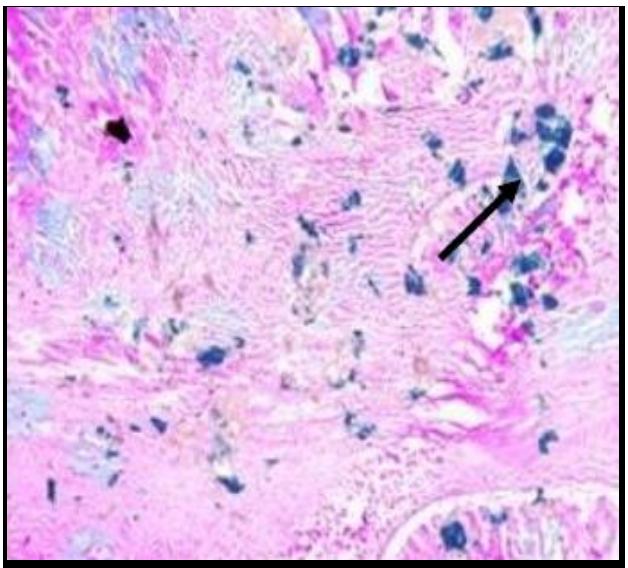

Figure 7 - Infected intestine stained with Ferric ferricyonide

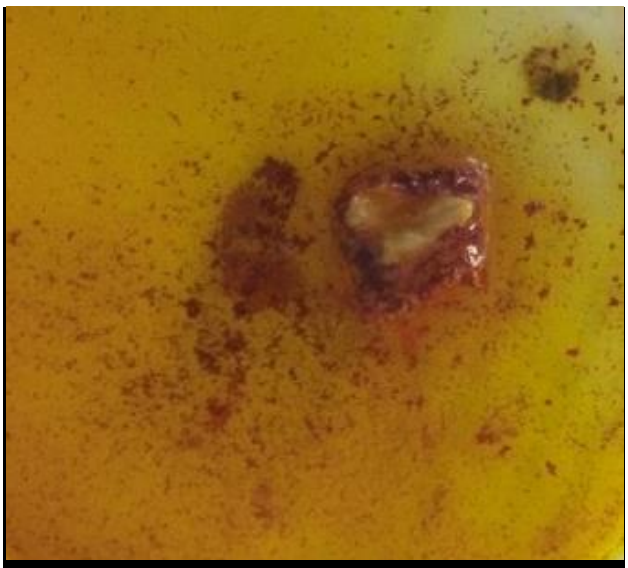

Figure 8 - Infected intestine stained with Free Aldehydes

CONCLUSION

The gastrointestinal tract is typically covered by mucus, the properties of which change in different regions of the alimentary canal. The mucus, which can be considered an aggregate secretion, is produced by mucous cells; mucus, however, differs from mucin which refers to specific glycoproteins within this secretion. Intestinal mucus, which is continuously secreted to renew the coating, is a dynamic system which is coupled to the immune system. An accelerated secretion is characterized by a rapid, massive mucous cell discharge in response to physiologic or pathologic stimuli by the parasite. Moreover, the histochemical investigations provide an insight into the nature of various physiological and pathological processes in the gastrointestinal tract occurred due to parasites. It has been observed that the different constituents are stimulated by particular parasite and particular loss in different organs of the digestive system of the fish studied. Histochemical study may provide a valuable with low cost effective tool for the diagnosis of diseases in histopathology, parasitic investigation and for the researchers in histopathology.

DECLARATIONS

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

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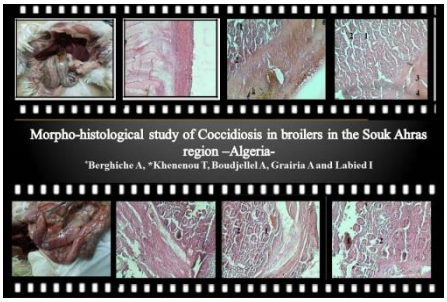

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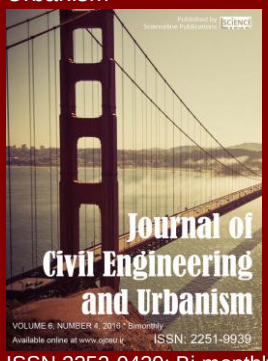

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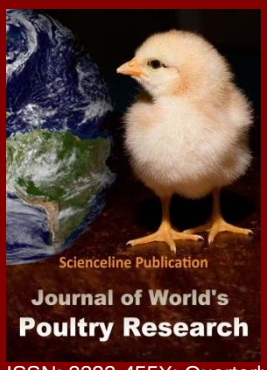

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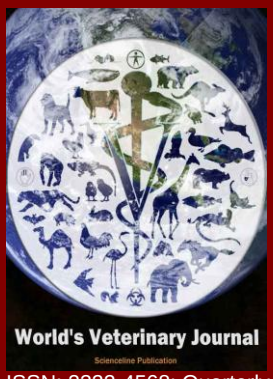

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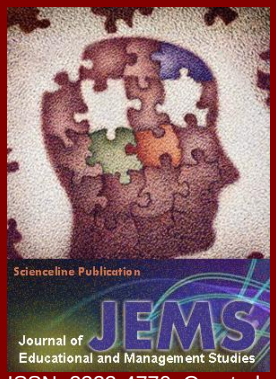

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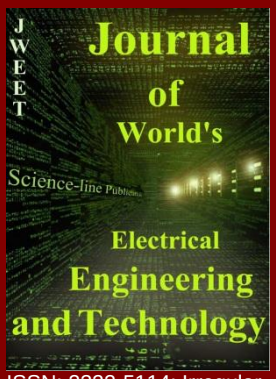

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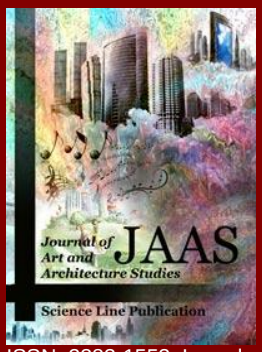

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