American Journal of Animal and Veterinary Sciences

Original Research Paper

Effect of a Mushroom (*Coriolus versicolor)* Based Probiotic on Goat Health

[1]Kingsley Ekwemalor, [1]Emmanuel Asiamah, [1]Sarah Adjei-Fremah and [2]Mulumebet Worku

[1]*Department of Energy and Environmental Systems*
[2]*Department of Animal Sciences*
North Carolina Agricultural and Technical State University, 1601 E Market Street, 27411, Greensboro, NC, USA

Article history
Received: 14-06-2016
Revised: 29-09-2016
Accepted: 04-10-2016

Corresponding Author:
Mulumebet Worku
Department of Animal Sciences, North Carolina Agricultural and Technical State University, 1601 E Market Street, 27411, Greensboro, NC, USA
Email: worku@ncat.edu

Abstract: Maintenance of an adequate immune response is essential to goat health. CorPet biomass is a mushroom (*Coriolus versicolor*) based probiotic used as an immune-stimulant in man, horses and small animals. Fifteen BoerXSpanish goats were used in a crossover design to evaluate the effect of a commercial mushroom based probiotic across two periods on resilience of goats to gastrointestinal parasites in pasture based systems. Following initial screening for infection, goats were assigned to three groups of five (n = 15) individuals. Powdered CorPet was soaked in hot or cold water and sterile filtered. Goats were drenched daily with 10 mL of the hot (treatment I) or cold (treatment II) extract daily for an 8 week period. A control group of five age-matched goats received sterile water (treatment III). Body Weight (BW), Fecal Egg Count (FEC), FAMACHA scores, Packed Cell Volume (PCV) and White Blood Cell Differential counts (WBC) were determined weekly. The concentration of total serum protein, 8 Pro-inflammatory cytokines and Prostaglandin (PGE2) secretion was evaluated using commercial ELISAs for each. Treatment had no effect on BW, FEC, FAMACHA scores, PCV and WBC. Total serum protein concentration ($p<0.001$) increased. Increased levels of interferon production regulator (IFNr), Rantes and Granulocyte-Colony Stimulating Factor (GCSF) and decreased levels of Granulocyte Macrophage Colony-Stimulating Factor (GM-CSF) were observed in treated goats. The concentration of PGE2 decreased in all groups over the study period of 8 weeks. Greater changes were observed in treated goats (65-80%) compared to control (50%). Thus in goats CorPet may modulate innate immunity through differential regulation of the secretion of serum proteins including cytokines and PGE2 to impact goat health.

Keywords: Goat, Immunity, Mushroom, Probiotic

Introduction

Goat production is a growing industry in the United States of America due to stable ethnic markets rapidly increasing demand for grass-fed or organically produced livestock and the growing popularity of specialty meat type goat breeds (Joshi *et al*., 2011). However, this production is negatively affected by several factors: Respiratory diseases such as pneumonia, feed toxicity, infectious and parasitic diseases. Goats are vulnerable to viral diseases, such as foot and mouth disease and bacterial diseases such as mastitis. Gastrointestinal Nematode (GIN) parasites are an important limiting factor in goat production systems globally, especially in humid climates (Rinaldi *et al*., 2007; Terrill *et al*., 2007). There are many parasites that cause an array of health problems in sheep and goat production, but none more significant than the nematode *Haemonchus contortus* and Coccidia (Miller and Horohov, 2006). Pathogenic coccidia in goats include *Eimeria arloingi*, *Eimeria christenseni* and *Eimeria ovinoidalis* (Orzechowski *et al*., 2012). At weaning, goats encounter lowered immune function, creating favorable conditions for an outbreak of diseases such as coccidiosis that can rapidly spread in a herd of flock, causing devastating consequences of diarrhea, dehydration and even death (Bowman, 2009).

In the United States and other countries around the world, resistance to current drugs by to gastrointestinal

nematodes and a lack of interest in developing new drugs by companies are challenges that are impacting small ruminant production (Zajac, 2002). Better treatment methods include the use of non-chemical alternatives, improved production practices and the use of genetics based breeding schemes. These approaches also include, inclusion of ingredients that modulate immune functions into animal feed to stimulate host defense (Gallois and Oswald, 2008; Hoste *et al*., 2008). Plant-based anthelmintic are being explored for use in the elimination of gastrointestinal parasites including extracts such as: Garlic, neem, wormwood, tobacco and Sericea Lespedeza (Worku *et al*., 2009; Worku *et al*., 2016a).

Mushrooms are widely appreciated and consumed for their nutritional value and medicinal properties (Wasser, 2002). Mushrooms are produced on natural materials from agriculture, woodlands, animal husbandry and manufacturing industries (Foluloke *et al*., 2014). Mushrooms are good sources of vitamins, minerals, proteins and carbohydrates in addition to their low levels of lipids and low caloric contents (Wasser, 2002; Kalac, 2013). They contain almost all the essential and non-essential amino acid with lysine as the most essential amino acid (Oei, 2005). Additionally, mushrooms are rich in many bioactive metabolites of high medicinal value such as lectins, polysaccharides, phenolics, terpenoids and volatile organic compounds (Zhou *et al*., 2007; Kalac, 2013). Mushroom extracts have been shown to exhibit immumomodulatory, antitumor/anticancer, antibacterial and antiviral, antioxidant and antihypoglycemic activities (Tsai *et al*., 2009). In human medicine, mushroom is also used as probiotics to modulate human health (Chang and Wasser, 2012). Research has shown the potential use of mushroom extracts as an additive to feed resource for livestock and as an alternative to antibiotic growth promoters in broiler chicken (Guo *et al*., 2005). There are reports on beneficial effects of mushroom, which are used as feed supplements and medicines in chickens (Ogbe *et al*., 2009). However, their use in treatment and or/prevention of diseases of economic importance in ruminants remains limited.

The objective of this study was to evaluate the effect of extracts of the edible mushroom CV on the resilience of goats to gastrointestinal parasites in pasture based systems.

Materials and Methods

Animals

Fifteen female BoerXSpanish goats (Worku *et al*., 2016b), from the goat herd at the North Carolina Agricultural and Technical University Small Ruminant Research Unit were used in this study. Animals were clinically healthy and not under any treatment. Initial sampling was carried out to determine the health of the animals. The study was approved by the Institutional Animal Care and Use Committee.

Housing and Feeding

Animals were maintained on pasture and housed in a shed overnight. The animals were supplemented with a commercial balanced feed (Southern States Quality kid and Goat Feed, Colfax, NC) which contained a high crude protein, crude fat, crude fiber, acid detergent fiber and fortified with vitamins and minerals such as calcium, phosphorus, copper, chloride, Selenium and Vitamin A. The goats were fed two pounds of feed per animal once a day. Water and hay were provided ad libitum.

Preparation of Mushroom Extracts

CorPet (CV) powder was purchased from Mycology Research Labs Ltd (United Kingdom). CorPet extract contains both the mycelium and the primodia (young fruit bodies) of *Coriolus versicolor* grown aseptically into a biomass. The biomass is then dried and milled into fine powder. Hot extracts (treatment I) was prepared by weighing 25 g of CorPet powder in 250 mL of sterile endotoxin free water and heating to 100°C with stirring for 20 min. Twenty-five (25) grams of CorPet was stirred in 250 mL of sterile endotoxin free distilled water and served as cold extract (treatment II). Endotoxin assay was done as previously described by (Adjei-Fremah *et al*., 2016). The extracts were left to cool and then stored at 4°C until it was used. Distilled water (treatment III) served as control.

Experimental Design

Fifteen BoerXSpanish goats were used in this study. The study involved a balanced crossover design (3 treatments x2 periods), with 4wk/period. There was no interval between periods. Experimental animals receiving control treatment (water) were maintained throughout the 8 weeks study period. Testing procedures were performed weekly and constituted of body weight, body condition scores, FAMACHA scores, Fecal Egg Count (FEC), Packed Cell Volume (PCV), white blood cell count, total plasma protein concentration, detection and secretion of cytokine Prostaglandin (PGE2).

CorPet Drench Administration

Ten (10) mL of the hot and cold extracts were given to each goat (5 goats per group) daily. Ten (10) mL of distilled water was administered to the control group daily for 60 days. Extracts were administered using a 10 mL syringe.

Sample Collection

Goats were weighed on a portable scale in kilograms before feeding in the morning. Body condition score was evaluated by physically assessing the rib areas using firm pressure with the fingers and running fingers down the goat's spine from the shoulders to the tail head and degree of fat cushion over these bones determines the score (Villaquiran *et al*.,

2004). Blood and fecal samples were collected and evaluated once a week throughout the experiment. Fecal samples were collected directly from the rectum of each animal. The Modified McMaster's technique (Whitlock, 1948) was used to measure fecal egg count. The number of strongyle eggs and coccidia oocyst were counted in duplicate, the average was calculated and then multiplied by 50 to get the Eggs Per Gram (EPG) of feces for each animal (Kaplan *et al*., 2004). The color of the conjunctival mucosa membranes of each animal was evaluated as classified into five categories according to the FAMACHA eye color chart (Kaplan *et al*., 2004). Blood samples (10 mL) were collected from the jugular vein into tubes containing ethylenediaminetetraacetic acid for cell count analysis and tubes containing Gel and Lithium Heparin (BD, Franklin Lakes, NJ) for serum collection. Blood samples were processed within 2 h of collection.

Packed Cell Volume (PCV) as a measure of anemia was evaluated using an aliquot of blood with anticoagulant. Blood was collected in micro-capillary tubes and centrifuged for 5 min at 14,000 rpm in an IEC MB Micro Hematocrit centrifuge (Damon/IEC Division). After centrifugation, samples were analyzed for percentage of packed cell volume using a micro-capillary reader (Damon/IEC Division).The white blood cell differential counts were determined as described by Schalm *et al*. (1975). White blood cell differential counts were performed using an Olympus B 201 microscope using a 100x magnification

Total serum protein concentration was evaluated from whole blood collected in Gel and Lithium Heparin tube as described by Adjei-Fremah *et al*. (2015). The tubes were centrifuged at 3600 rpm for 20 min to separate serum from blood cells. Serum was stored at -70°C until further analysis. The Pierce^{TM} BCA Assay kit (Rockford, IL) was used to determine the total protein concentration in serum as recommended by the manufacturer.

The concentration of 8 pro-inflammatory cytokines was assessed using the Human Inflammation ELISA kit (Signosis Inc, Santa Clara, CA) as described by Worku *et al*. (2016a). The cytokines assayed included Tumor Necrosis Factor-alpha (TNF-α), interferon regulator (IFNr), Granulocyte-Colony Stimulating Factor (G-CSF), Granulocyte Macrophage-Colony Stimulating Factor (GM-CSF), interleukin-1a (IL-1a), interleukin-8 (IL-8), interferon inducible protein 10 (IP-10) and Regulated on Activation, Normal T Cell Expressed and Secreted (RANTES). Serum samples were pooled together and assayed in triplicate for each treatment group for week 0, 4 and 8.

The concentration of prostaglandin E2 (PGE2) was measured in triplicate samples from treated and control groups using a commercial Enzyme-linked immunosorbent assay kit (Cayman Chemical, An Arbour, MI) as described by Asiamah *et al*. (2016).

Statistical Analysis

Data was analyzed with SAS using a mixed model ANOVA with repeated measures (Cody and Smith, 1997; SAS Institute Cary, NC), with treatment, periods and the interaction of treatment and periods as the fixed effects and goats receiving treatment as the random effect. Results were considered statistically significant if $p<0.05$. Each goat received both treatments; period was included in the model for analyses. A Pearson correlation and coefficient was used to determine correlation between variables.

Results

All animals were in good health at the beginning and end of the experiment. In addition, no clinically significant differences were found in clinical indexes, including vital signs of anemia, before and after administration of mushroom extracts.

Effect of Mushroom Supplementation on Body Weight

Goats used in this experiment had an initial average weight of >52 kg across all groups. There was no significant difference in body weight between treatment and control groups ($p<0.1045$) at the end of the study. There was no effect of CorPet mushroom on body weight. The average mean weight for control was 66.1 kg (Table 1), CorPet treatment resulted in a mean weight of 62.7 kg in the treated animals. Repeated measures analysis showed that body weight increased over time. Correlation between body weight and lymphocyte cell count ($p<0.028$) was observed. At week 4, there was an observable trend. The goats receiving the cold extract had the lower body weight gain (58 kg) compared to the other two groups (61 and 66 kg) and remained the same when treatments were switched.

Effect of Mushroom Supplementation on Body Condition

CorPet mushroom probiotic had no effect on body condition. There was no significant difference in body condition scores between animals in the control and treatment groups (Table 1).

Effects of Mushroom Supplementation on Indicators of Infection Fecal Egg Count

Strongyle eggs and coccida oocytes were detected in fecal samples indicating infection. A mean number of 350 strongyle epg and an average of 114 cocidia oocyst were recorded across treatments. There was no significant difference between control and treatment groups (Fig. 1a). There was no effect of CorPet extracts on the coccidia oocyst count. Strongyle *sp* egg count and Coccidia oocyst count started off high but were lower after the first 4 weeks in treated and control animals (Fig. 1b).

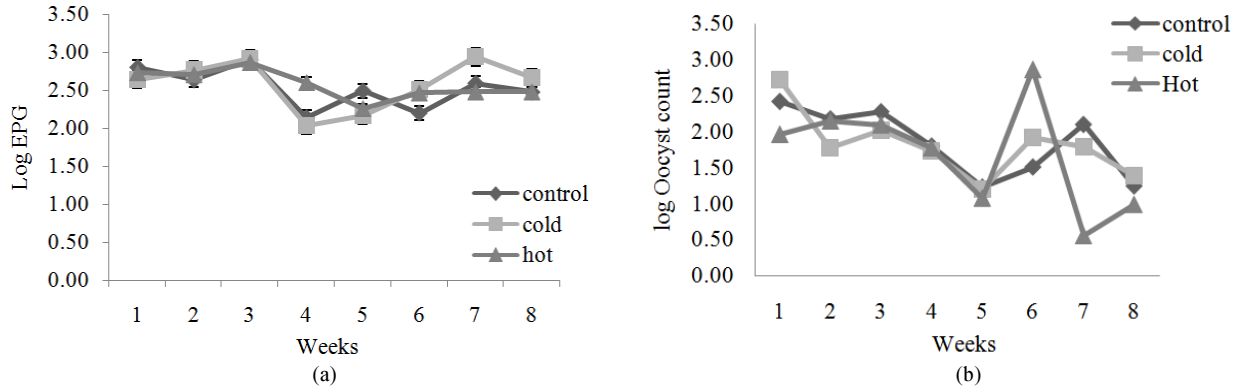

Fig. 1. (a) The Effect of CorPet Extracts on Haemochus contortus eggs per Gram feces over a period of 8 weeks, (b) The Effect of CorPet Extracts on coccidia oocyst per gram feces over a period of 8 weeks

Table 1.Mean and standard error of Body Weight (BW), Body Condition (BC), Famacha and Packed Cell Volume (PCV) of goats based on the different treatments

	Control			Cold			Hot		
Parameter	Wk1	Wk4	Wk8	Wk1	Wk4	Wk8	Wk1	Wk4	Wk8
BW(kg)	52.8±7.4	68.6±3.6	76.4±4.0	54.2±4.9	62.8±5.8	62.8±5.8	52.0±12.7	51.4±6.3	74±5.4
BC	2.0±0.48	3.0±0.2	3.0±0.1	2.0±0	3.0±0	2.0±0	2.0±0	2.0±0	2.0±0
FAMACHA	3.0±0.5	3.2±0.2	2.0±0	2.4±0.2	2.6±0.2	2.2±0.2	3.0±0.9	2.6±0.4	2.0±0
PCV, %	29.8±3.8	30.5±1.8	28.7±2.0	29.3±5.9	26.7±1.6	26.9±1.7	29.7±7.3	26.5±2.2	26.7±0.8

Effect of Mushroom Supplementation on Indicators of Anemia FAMACHA

In this study FAMACHA scores were in the range of red (FAMACHA = 1) to red-pink (FAMACHA = 2) indicating that anemia was generally not a major problem in the study population. FAMACHA scores were similar between control and treatment groups (Table 1).

Effect of Mushroom on Packed Cell Volume

Packed cell volume levels were within the normal range for all goats throughout the study. There was no significant difference between treated and control animals. However, there was a time effect for the first four weeks ($p<0.05$) and the last four weeks ($p<0.002$) which may be associated with infection as the animals were on pasture. On a weekly base the treatment group had a lower PCV compared to control. There was no correlation between FAMACHA scores and PCV; however both were in the normal range for anemia (Table 1).

Effect of Mushroom Supplementation on Indicators of Immunity: White Blood Cell Differential Count

There was no significant difference in the percentages of neutrophils (34%), lymphocytes (53%), monocytes (5%), basophils (5%) and eosinophils (1%) between treatments and control groups. Neutrophil cell count increased early and over time there was a significant increase of lymphocyte count ($p<0.007$) and a decrease in neutrophil count ($p<0.001$) during the last four weeks (Fig. 2). There was a significant increase observed in eosinophil count in last four weeks ($p<0.01$).

Total Serum Protein Concentration

There was a significant increase in total serum protein concentration in treated goats compared to the control ($p<0.001$). Serum protein concentration increased significantly ($p<0.001$) for the first four weeks and decreased in the last four weeks ($p<0.001$) in all groups. In weeks 2 through 5, treated goats had a higher serum protein concentration compared to the control group (Fig. 3).

Secretion of Pro-Inflammatory Cytokines in Serum

Pro-inflammatory cytokine levels were measured in serum from the treatment and control groups at week 0, 4 and 8 of the study. The cytokines TNF-α, IL-1a and IL-8 were not detected. Animals that received CV extract expressed increased levels of IFNr (54%), Rantes (94%) and GCSF (64%) and decreased levels of GM-CSF (6%) when the concentration of cytokines at week 1 was compared to levels at week 8 (Fig. 4).

Secretion of PGE2

The concentration of PGE2 decreased in all groups over the study period of 8 weeks. Greater changes were observed in treated goats (65-80%) compared to control (50%) (Fig. 5).

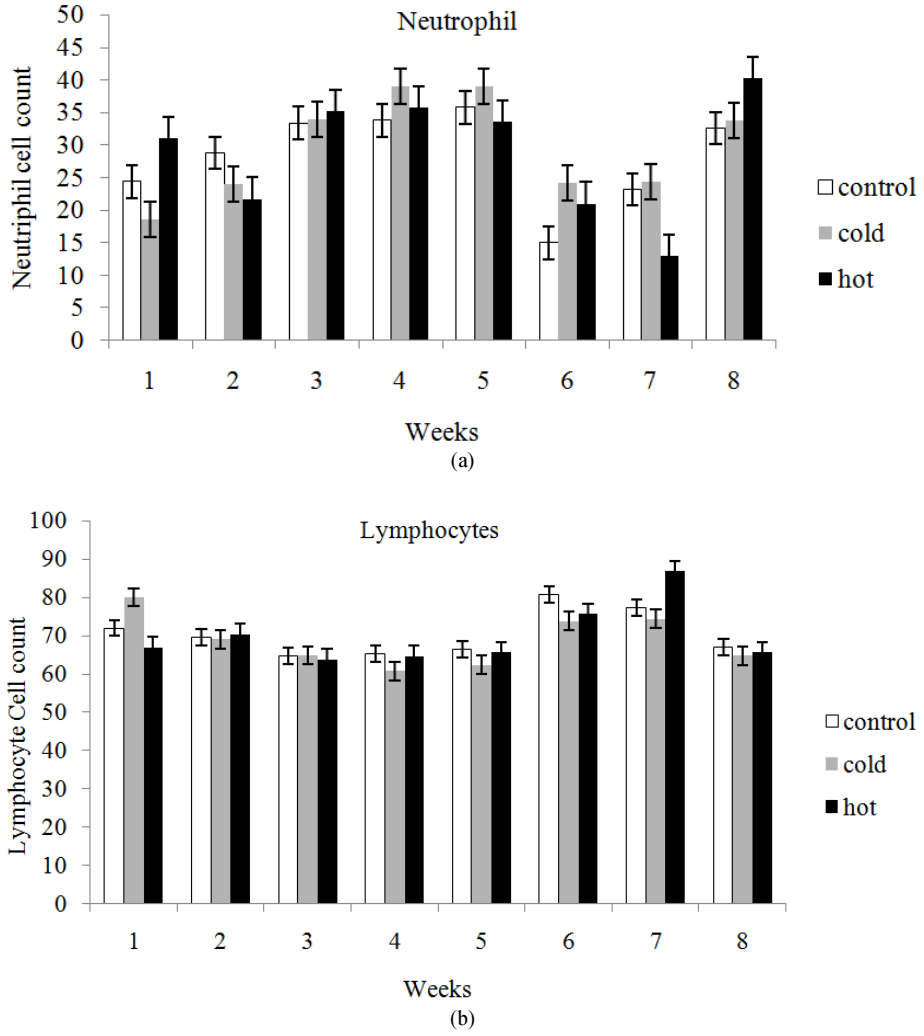

Fig. 2. (a) The Effect of CorPet Extracts on neutrophil count over a period of 8 weeks, (b) The Effect of CorPet Extracts on lymphocyte count over a period of 8 weeks

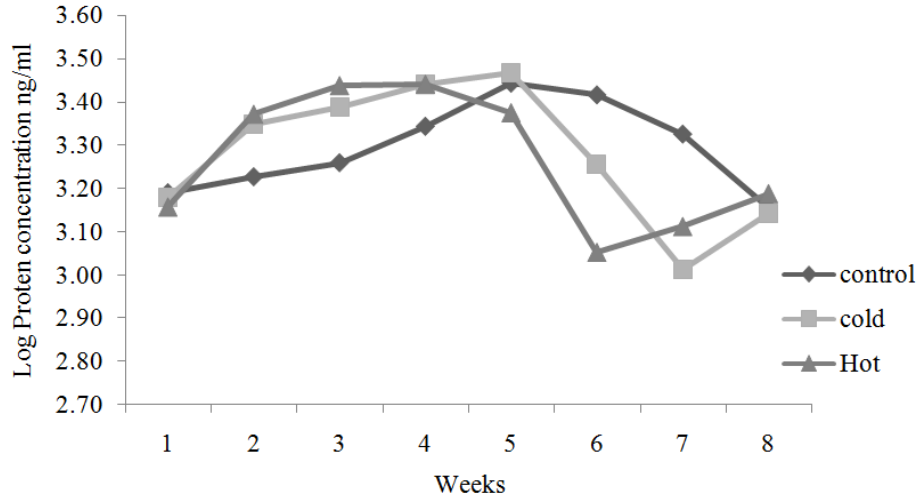

Fig. 3. Effect of CorPet mushroom extracts on serum protein concentration in ng/µL over a period of 8 weeks

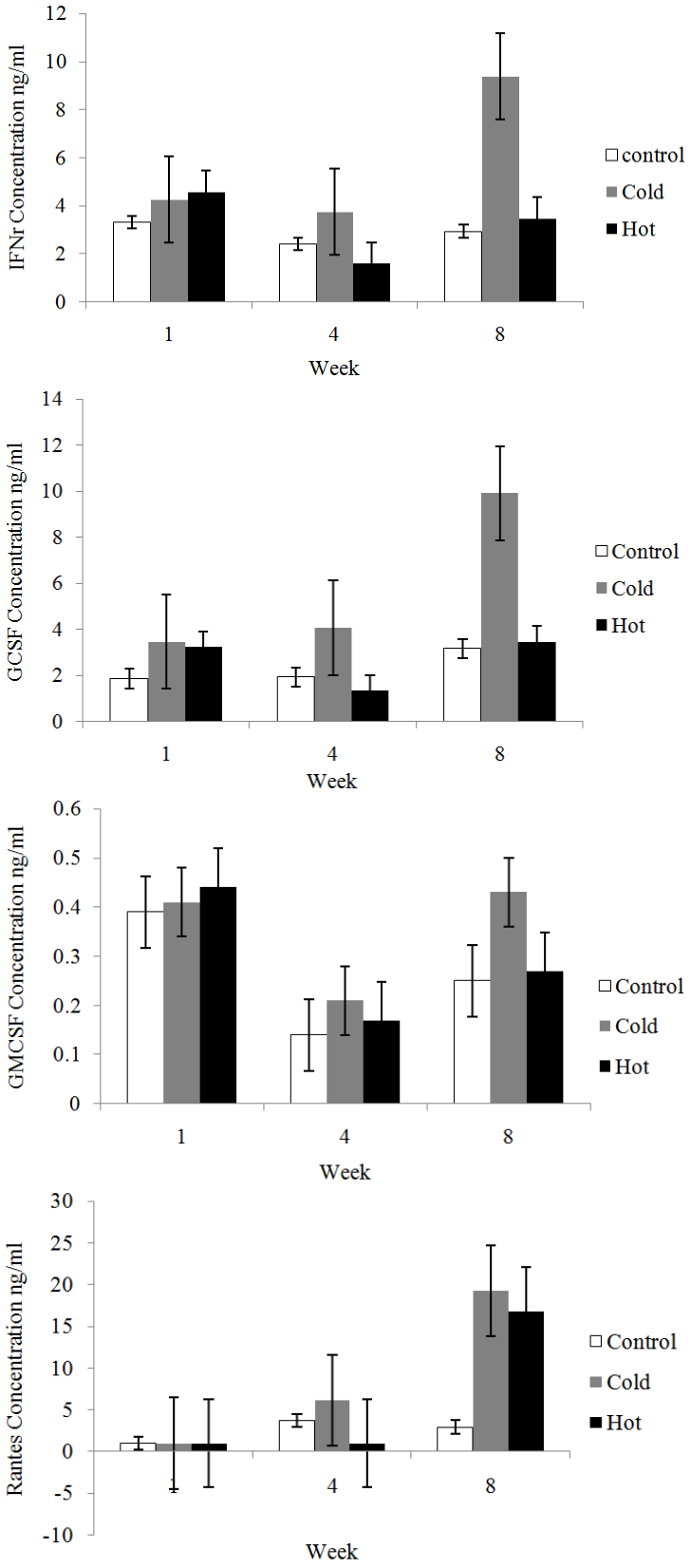

Fig. 4. Effect of CorPet on the secretion of cytokines on day 1, week 4 and week 8

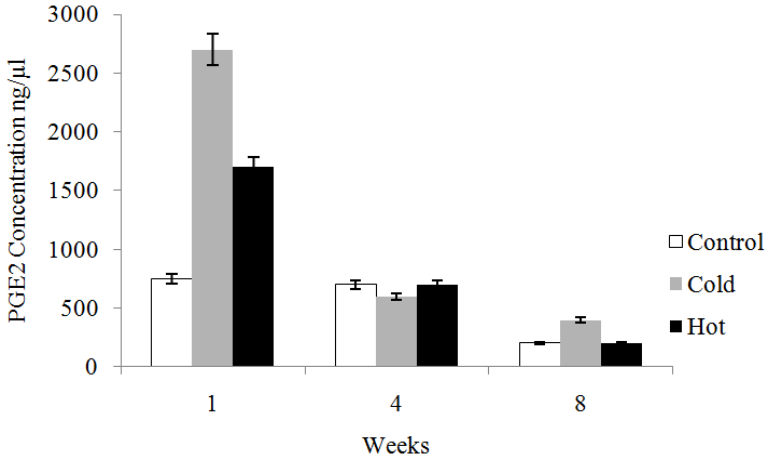

Fig. 5. Effect of Corpet on the secretion of PGE2 showing error bars with percentage

Discussion

Use of probiotics in animal production may offer an alternative approach to control diseases by boosting the host's resilience. Mushrooms and mushroom components have been reported to have myriad of positive health benefits, mainly on the basis of *in vitro* and *in vivo* animal trials (Roupas *et al*., 2012). Studies have shown their potential as feed additives in livestock. They have been studies as alternatives to the use of probiotics in poultry production. Mushrooms and their extracts are generally well-tolerated.

In this study CV did not have an effect on body weight, body condition, FAMACHA scores and PCV which remained within the normal range of healthy goats. Body weight is an important parameter in determining the health status of an animal over a certain period of time. A decline in weight can be associated with parasitic infection, high temperatures that stress the animal and also lack of appropriate nutrition. It is expected that animals keep a relatively consistent body weight during growth. Healthy normal adult female Boer goats have a body weight that ranges from 55 to 90 kg (Coffey *et al*., 2002). Goats used in this study were 3 weeks after weaning and an increase in weight was expected across all treatments. Diet and feed were kept constant during the experimental period in other to rule out any source of variation. Animal studies have shown that mushroom enriched diet have no effect on body weight (Handayani *et al*., 2011). Whitley *et al*. (2009) reported that a commercial probiotic had no effect on body weight of goats as observed in treated animals in our study. Daneshmand *et al*. (2011) showed that supplementation of *Pleurotus ostreatus* had no effect on weight gain in mice during the entire experiment.

Supplementation with CV did not impact goat body condition scores. Body condition scoring is used to achieve proper management of body reserves and has proved to be an effective and easy to use method to assess the impact of parasite infection (Mendizabal *et al*., 2011). In this study, body condition scores were in the range of 2 to 3 indicating a healthy body condition (Mendizabal *et al*., 2011). There was no effect of treatment on both indicators of anemia. Packed cell volume is an important tool in the diagnosis of parasitic infection (Vatta *et al*., 2001). The PCV obtained in this study was within the range for a healthy goat. A normal PCV for an adult goat ranges from 24 to 48% (Karki, 2008). Results obtained from this study were similar to the study by Ogbe *et al*. (2009) in mice indicating that aqueous mushroom extract of *Ganoderma lucidum* had no effect on PCV. High levels of *Haemonchus contortus* FEC are associated with anemia which can be accurately assessed using the FAMACHA© system (Kaplan *et al*., 2004; Mahieu *et al*., 2007; Scheuerle *et al*., 2010) on farm and by assessment of PCV in the lab. Low PCV may be indicative of anemia, which is an undesirable condition that points to a compromised health status of the animal. Kaplan *et al*. (2004) and Burke *et al*. (2007) reported a correlation between PCV and FEC but there was no correlation between FEC and PCV in this study. This may be due to the low levels of infection in this study.

There was no effect of treatment on FEC. The detection of parasites eggs in fecal samples indicates animal were infected. Fecal egg count is a parameter widely used to determine parasitic infection in animals (Kaplan *et al*., 2004). *Strongyliodes* species is considered to be one of the most pathogenic gastrointestinal parasitic nematode in small ruminants (Nwosu *et al*., 2007). The strongyle egg counts were not very high. The coccidia oocyst counts were also low. Guo *et al*. (2005) reported that mushroom extracts of *Lentinus edodes* and *Tremella fuciformis* showed low fecal oocyst output. It has been reported that mushroom (*Ganoderma lucidum*) in broilers infected with *Eimeria tenella* reduced the

number of *Eimeria tenella* oocysts shed in the feces and led to improved weight gain (Ogbe *et al*., 2009).

The total WBC count is a tool to evaluate the peripheral immune response (Kaplan *et al*., 2004). Goats have 34% neutrophils, 53% lymphocytes, 1% basophils, 5% monocytes and 5% eosinophils (Kahn and Line, 2010) in peripheral blood. Statistically, there was no effect of CorPet on differential white blood cell count. However, there were changes in neutrophil to lymphocyte ratio at the beginning and towards the end of the experiment. This initial increase in neutrophils may be an indicator of activation of innate immunity. Also increase in lymphocyte count indicates a hightened level of immune status and may be attributed to stress. Kyakulaga *et al*. (2013), reported that aqueous extracts of *Auricularia* sp and *Pleurotus* sp increased total and differential WBC counts in immunosuppressed Wistar rats.

There was an increase in serum protein concentration which indicates that extracts of CV increased protein translation which is consistent with the findings of Johnson *et al*. (2009), who reported that immunomodulation by mushroom probiotics may stimulate serum proteins.

The release of proinflammatory cytokines is essential for host survival from infection and is also required for tissue repair. The pro-inflammatory cytokines TNF, IL-1a and IL-8 were secreted in small amounts in both control and treated groups. This may be attributed to low concentrations or time of sampling as these cytokines are short lived and expressed in early cell activation. Furthermore studies by Lim (2011) show that the anti-inflammatory response with CV extract correlated with the reduced expression of TNF-α, IL-1β and IL-6 which corresponds to the low secretion of TNF-α obtained in this study. Yu *et al*. (2009) also reported a decrease in levels of serum mucosal interleukin-2 (IL-2) and TNF-α in rats following oral ulceration of *Lentinus edodes.* Johnson *et al*. (2009) reported that *in vivo* study of *Agaricus blazei* reduced the levels of TNF-α by 84% and IL-2 by 46%. However, Seong and Kim (2010) reported that when cells were stimulated with β-glucan the macrophage cells increased TNF-α expression, however, in our study there was no secretion of TNF-α.

Treatment with CV extracts modulated the levels of GCSF, Rantes and GMCSF. The growth function of GCSF and GMCSF are associated with the release of granulocytes and may contribute to the observed changes in white blood differential counts relative to lymphocytes and monocytes counts. Batbayar *et al*. (2011) have also reported that β-glucan from *Ganoderma lucidum* induced the secretion of GCSF and Rantes. It has been observed that β-glucan activates B-lymphocytes and macrophages through Toll-like receptors, modulating the immune system and inducing the production of cytokines (Liao *et al*., 2004). The antioxidant activity of CV and the limited adsorption of its polysaccaridepeptide and polysaccaridekrestin across the intestinal mucosa to the blood have been reported. Chang *et al*. (2010) reported that oral administration of Enoki mushroom displayed anti-tumor activity through activating both innate and adaptive immunity of the host to prime a cytotoxic immune response and IFN-γ played a key role in the anti-tumor efficacy. Thus in goats, oral administration of CV stimulated cytokine production.

Prostagladin E2 is an inflammatory mediator that is secreted by various cells. Jedinak *et al*. (2011) reported that concentrates of oyster mushroom inhibited production of PGE2 through the down regulation of COX-2 expression. The increased percentage reduction observed in treated goats may indicate that CV has a similar effect and needs further study at the level of gene expression.

Conclusion

The results of this study show that extracts of the mushroom *Coriolus versicolor* were not harmful to goat health and production. The *CV* extracts modulate innate immunity by increasing serum protein concentration, selectively modulating pro-inflammatory cytokine and PGE2 secretion. Thus, results from this study suggest that mushroom based probiotics such as CorPet could be safe and effective for enhancing goat immunity as supplements in goat feed. This study provides an insight on the mechanism of action of mushroom based probiotic to enhance goat innate immunity and thus warrants further study.

Acknowledgement

We appreciate the assistance of Gary Summers and Hamid Ismail during sample collection and Tiffany Martin, Jordan Page and Allie McMahan for their assistance in this project. Funding support was from USDA project NC.X-271-5-13-120-1, Modulation of Receptor Cross-Talk for cattle, Sheep and Goat Innate Immunity.

Author's Contributions

Kingsley Ekwemalor: Design and implementation of research, conducted research work, data analysis of research data and manuscript write up for publication.

Emmanuel Asiamah: Assisted with the study plan, data interpretation and manuscript write up.

Sarah Adjei-Fremah: Assisted with the study plan, data interpretation and manuscript write up.

Mulumebet Worku: Designed and supervised the research, data interpretation and manuscript write up.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of

other authors read and approved the manuscript and no ethical issues involved.

References

Adjei-Fremah, S., E.K. Asiamah, K. Ekwemalor, L. Jackai and K. Schimmel *et al*., 2016. Modulation of bovine wnt signaling pathway genes by cowpea phenolic extract. J. Agric. Sci., 8: 21-35. DOI: 10.5539/jas.v8n3p21

Adjei-Fremah, S., L.E. Jackai and M. Worku, 2015. Analysis of phenolic content and antioxidant properties of selected cowpea varieties tested in bovine peripheral blood. Am. J. Animal Vet. Sci., 10: 235-245. DOI: 10.3844/ajavsp.2015.235.245

Asiamah, E.K., S. Adjei-Fremah, B. Osei, K. Ekwemalor and M. Worku, 2016. An extract of sericea lespedeza modulates production of inflammatory markers in Pathogen Associated Molecular Pattern (PAMP) activated ruminant blood. J. Agric. Sci., 8: 1-11. DOI: 10.5539/jas.v8n9p1

Batbayar, S., M.J. Kim and H.W. Kim, 2011. Medicinal Mushroom Lingzhi or Reishi, Ganoderma lucidum (W.Curt.:Fr.) P. Karst., β-Glucan Induces Toll-like Receptors and Fails to Induce Inflammatory Cytokines in NF-κB Inhibitor-Treated Macrophages. Int. J. Med. Mushrooms, 13: 213-225. DOI: 10.1615/IntJMedMushr.v13.i3.10

Bowman, D.D., 2009. Georgis' Parasitology for Veterinarians. 1st Edn., Elsevier Health Sciences, St. Louis, ISBN-10: 1416044124, pp: 451.

Burke, J.M., R.M. Kaplan, J.E. Miller, T.H. Terrill and W.R. Getz *et al*., 2007. Accuracy of the FAMACHA system for on-farm use by sheep and goat producers in the southeastern United States. Vet. Parasitol., 147: 89-95. DOI: 10.1016/j.vetpar.2007.03.033

Chang, H.H., K.Y. Hsieh, C.H. Yeh, Y.P. Tu and F. Sheu, 2010. Oral administration of an Enoki mushroom protein FVE activates innate and adaptive immunity and induces anti-tumor activity against murine hepatocellular carcinoma. Int. Immunopharmacol., 10: 239-246. DOI: 10.1016/J.INTIMP.2009.10.017

Chang, S.T. and S.P. Wasser, 2012. The role of culinary-medicinal mushrooms on human welfare with a pyramid model for human health. Int. J. Med. Mushrooms, 14: 95-134. DOI: 10.1615/IntJMedMushr.v14.i2.10

Cody, R.P. and J.K. Smith, 1997. Repeated measures designs. Applied Statistics and the SAS Programming Language.

Coffey, K.P., W.K. Coblentz, T.G. Montgomery, J.D. Shockey and K.J. Bryant *et al*., 2002. Growth performance of stocker calves backgrounded on sod-seeded winter annuals or hay and grain. J. Animal Sci., 80: 926-932. DOI: 10.2527/2002.804926x

Daneshmand, A., G.H. Sadeghi, A. Karimi and A. Vaziry, 2011. Effect of oyster mushroom (*Pleurotus ostreatus*) with and without probiotic on growth performance and some blood parameters of male broilers. Animal Feed Sci. Technol., 170: 91-96. DOI: 10.1016/J.ANIFEEDSCI.2011.08.008

Foluloke, A., O. Adedokun and O. Alabi, 2014. Assessing spent mushroom substrate as a replacement to wheat bran in the diet of broilers.

Gallois, M. and I.P. Oswald, 2008. Immunomodulators as efficient alternatives to in -feed antimicrobials in pig production? Arch Zootechn, 11: 15-32.

Guo, F.C., R.P. Kwakkel, B.A. Williams, X. Suo, W.K. Li and M.W.A. Verstegen, 2005. Coccidiosis immunization: Effects of mushroom and herb polysaccharides on immune responses of chickens infected with *Eimeria tenella*. Avian Diseases, 49: 70-73. DOI: 10.1637/7227-062504R1

Handayani, D., J. Chen, B.J. Meyer and X.F. Huang, 2011. Dietary Shiitake mushroom (*Lentinus edodes*) prevents fat deposition and lowers triglyceride in rats fed a high-fat diet. J. Obesity. DOI: 10.1155/2011/258051

Hoste, H., J.F.J. Torres-Acostat and A.J. Aguilar-Caballero, 2008. Nutrition–parasite interactions in goats: Is immunoregulation involved in the control of gastrointestinal nematodes? Parasite Immunol., 30: 79-88. DOI: 10.1111/j.1365-3024.2007.00987.x

Jedinak, A., S. Dudhgaonkar, Q.L. Wu, J. Simon and D. Sliva, 2011. Anti-inflammatory activity of edible oyster mushroom is mediated through the inhibition of NF-κB and AP-1 signaling. Nutrit. J., 10: 52-52. DOI: 10.1186/1475-2891-10-52

Johnson, E., D.T. Førland, L. Saetre, S.V. Bernardshaw and T. Lyberg *et al*., 2009. Effect of an extract based on the medicinal mushroom Agaricus blazei Murill on release of cytokines, chemokines and leukocyte growth factors in human blood *ex vivo* and *in vivo*. Scandinavian J. Immunol., 69: 242-250. DOI: 10.1111/j.1365-3083.2008.02218.x

Joshi, B.R., D.S. Kommuru, T.H. Terrill, J.A. Mosjidis, J.M. Burke and K.P. Shakya *et al*., 2011. Effect of feeding sericea lespedeza leaf meal in goats experimentally infected with *Haemonchus contortus*. Vet. Parasitol., 178: 192-197. DOI: 10.1016/J.VETPAR.2010.12.017

Kahn, C.M. and S. Line, 2010. The Merck Veterinary Manual. 10th Edn., Wiley, Rahway, NJ, ISBN-10: 091191093X, pp: 2945.

Kalac, P., 2013. A review of chemical composition and nutritional value of wild-growing and cultivated mushrooms. J. Sci. Food Agric., 93: 209-218. DOI: 10.1002/jsfa.5960

Kaplan, R.M., J.M. Burke, T.H. Terrill, J.E. Miller and W.R. Getz *et al*., 2004. Validation of the FAMACHA© eye color chart for detecting clinical anemia in sheep and goats on farms in the southern United States. Vet. Parasitol., 123: 105-120. DOI: 10.1016/j.vetpar.2004.06.005

Karki, K., 2008. A laboratory epidemiological outbreak investigation of kumri (*Cerebrospinal nematodiasis*) and use of diethylecarbamazin in treatment of Goat in Banke district of Mid-Western Region of Nepal. Vet. World, 1: 168-170.

Kyakulaga, A.H., P.E. Ogwang, C. Obua, G. Nakabonge and E.N. Mwavu, 2013. Immunomodulatory Effects of aqueous extracts of auricularia sp and pleurotus sp mushrooms in cyclophosphamide-immunosuppressed wistar rats. Brit. J. Pharmaceutical Res., 3: 662-670. DOI: 10.9734/BJPR/2013/1994

Liao, S.C., Y.C. Cheng, Y.C. Wang, C.W. Wang and S.M. Yang *et al*., 2004. IL-19 induced Th2 cytokines and was up-regulated in asthma patients. J. Immunol., 173: 6712-6718. DOI: 10.4049/jimmunol.173.11.6712

Lim, B.O., 2011. *Coriolus versicolor* suppresses inflammatory bowel disease by inhibiting the expression of STAT1 and STAT6 associated with IFN-γ and IL-4 expression. Phytotherapy Res., 25: 1257-1261. DOI: 10.1002/ptr.3378

Mahieu, M., R. Arquet, T. Kandassamy, N. Mandonnet and H. Hoste, 2007. Evaluation of targeted drenching using Famacha© method in Creole goat: reduction of anthelmintic use and effects on kid production and pasture contamination. Vet. Parasitol., 146: 135-147. DOI: 10.1016/j.vetpar.2007.02.003

Mendizabal, J.A., R. Delfa, A. Arana and A. Purroy, 2011. Body condition score and fat mobilization as management tools for goats on native pastures. Small Ruminant Res., 98: 121-127. DOI: 10.1016/j.smallrumres.2011.03.029

Miller, J.E. and D.W. Horohov, 2006. Immunological aspects of nematode parasite control in sheep. J. Animal Sci., 84: E124-E132. PMID: 16582083

Nwosu, C.O., P.P. Madu and W.S. Richards, 2007. Prevalence and seasonal changes in the population of gastrointestinal nematodes of small ruminants in the semi-arid zone of north-eastern Nigeria. Vet. Parasitol., 144: 118-124. DOI: 10.1016/J.VETPAR.2006.09.004

Oei, P., 2005. Small-scale mushroom cultivation: Oyster, shiitake and wood ear mushrooms. Agromisa, 90: 8573-038.

Ogbe, A.O., S.E. Atawodi, P.A. Abdu, A. Sannusi and A.E. Itodo, 2009. Changes in weight gain, faecal oocyst count and packed cell volume of Eimeria tenella-infected broilers treated with a wild mushroom (Ganoderma lucidum) aqueous extract. J. South African Vet. Med. Associat., 80: 97-102. DOI: 10.4102/jsava.v80i2.179

Orzechowski, K.L., M.D. Swain, M.G. Robl, C.A. Tinaza and H.L. Swaim *et al*., 2012. Neurotoxic effects of ivermectin administration in genetically engineered mice with targeted insertion of the mutated canine ABCB1 gene. Am. J. Vet. Res., 73: 1477-1484. DOI: 10.2460/ajvr.73.9.1477

Rinaldi, L., V. Veneziano and G. Cringoli, 2007. Dairy goat production and the importance of gastrointestinal strongyle parasitism. Trans. Royal Society Tropical Med. Hygiene, 101: 745-746. DOI: 10.1016/j.trstmh.2007.03.010

Roupas, P., J. Keogh, M. Noakes, C. Margetts and P. Taylor, 2012. The role of edible mushrooms in health: Evaluation of the evidence. J. Functional Foods, 4: 687-709. DOI: 10.1016/J.JFF.2012.05.003

Schalm, O.W., N.C. Jain and E.J. Carrol, 1975. Veterinary haematology. Lea Febiger Philadelphia USA.

Scheuerle, M., M. Mahling, J. Muntwyler and K. Pfister, 2010. The accuracy of the FAMACHA©-method in detecting anaemia and haemonchosis in goat flocks in Switzerland under field conditions. Vet. Parasitol., 170: 71-77. DOI: 10.1016/j.vetpar.2010.01.035

Seong, S.K. and H.W. Kim, 2010. Potentiation of innate immunity by β-glucans. Mycobiology, 38: 144-148. DOI: 10.4489/MYCO.2010.38.2.144

Terrill, T.H., J.A. Mosjidis, D.A. Moore, S.A. Shaik and J.E. Miller *et al*., 2007. Effect of pelleting on efficacy of sericea lespedeza hay as a natural dewormer in goats. Vet. Parasitol., 146: 117-122. DOI: 10.1016/j.vetpar.2007.02.005

Tsai, S.Y., S.J. Huang, S.H. Lo, T.P. Wu and P.Y. Lian *et al*., 2009. Flavour components and antioxidant properties of several cultivated mushrooms. Food Chem., 113: 578-584. DOI: 10.1016/J.FOODCHEM.2008.08.034

Vatta, A.F., B.A. Letty, M.J. Van der Linde, E.F. Van Wijk and J.W. Hansen *et al*., 2001. Testing for clinical anaemia caused by *Haemonchus* spp. in goats farmed under resource-poor conditions in South Africa using an eye colour chart developed for sheep. Vet. Parasitol., 99: 1-14. DOI: 10.1016/S0304-4017(01)00446-0

Villaquiran, M., T.A. Gipson, R.C. Merkel, A.L. Goetsch and T. Sahlu, 2004. Body condition scores in goats. American Institute for Goat Research, Langston University.

Wasser, S., 2002. Medicinal mushrooms as a source of antitumor and immunomodulating polysaccharides. Applied Microbiol. Biotechnol., 60: 258-274. DOI: 10.1007/s00253-002-1076-7

Whitley, N.C., D. Cazac, B.J. Rude, D. Jackson-O'Brien and S. Parveen, 2009. Use of a commercial probiotic supplement in meat goats. J. Animal Sci., 87: 723-728. DOI: 10.2527/jas.2008-1031

Whitlock, H.V., 1948. Some modifications of the McMaster helminth egg-counting technique and apparatus. J. Council Sci. Industrial Res., 21: 177-180.

Worku, M., A. Abdalla, S. Adjei-Fremah and H. Ismail, 2016a. The impact of diet on expression of genes involved in innate immunity in goat blood. J. Agric. Sci., 8: 1-9. DOI: 10.5539/jas.v8n3p1

Worku, M., A. Abdalla, S. Ibrahim, S. Adjei-Fremah and H. Ismail, 2016b. The impact of a sericea lespedeza diet on bifidobacteria in goat rumen. Int. J. plant, Animal Environ. Sci.

Worku, M., R. Franco and J.H. Miller, 2009. Evaluation of the activity of plant extracts in Boer goats. Am. J. Animal Vet. Sci., 4: 72-79. DOI: 10.3844/ajavsp.2009.72.79

Yu, Z., Y. LiHua, Y. Qian and L. Yan, 2009. Effect of *Lentinus edodes* polysaccharide on oxidative stress, immunity activity and oral ulceration of rats stimulated by phenol. Carbohydrate Polymers, 75: 115-118. DOI: 10.1016/J.CARBPOL.2008.07.002

Zajac, A., 2002. Controlling goat parasites -- is it a losing battle? College of Veterinary Medicine.

Zhou, L., I.I. Ivanov, R. Spolski, R. Min and K. Shenderov *et al*., 2007. IL-6 programs T_H-17 cell differentiation by promoting sequential engagement of the IL-21 and IL-23 pathways. Nature Immunol., 8: 967-974. DOI: 10.1038/ni1488