

Journal of Veterinary Medicine and Animal Health

Volume 5 Number 11, November 2013



*Academic
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Cole (2000), Steddy et al. (2003), (Kelebeni, 1983), (Bane and Jake, 1992), (Chege, 1998; Cohen, 1987a,b;Tristan, 1993,1995), (Kumasi et al., 2001)

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Charnley AK (1992). Mechanisms of fungal pathogenesis in insects with particular reference to locusts. In: Lomer CJ, Prior C (eds), Pharmaceutical Controls of Locusts and Grasshoppers: Proceedings of an international workshop held at Cotonou, Benin. Oxford: CAB International. pp 181-190.

Jake OO (2002). Pharmaceutical Interactions between *Striga hermonthica* (Del.) Benth. and fluorescent rhizosphere bacteria Of *Zea mays*, L. and *Sorghum bicolor* L. Moench for Striga suicidal germination In *Vigna unguiculata*. PhD dissertation, Tehran University, Iran.

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ARTICLES

Review

Probiotics in animal production: A review 308
Ezema, C.

A review of the role of five kinds of alternatives to in-feed antibiotics in broiler production 317
Rozbeh Fallah, Ali Kiani and Arash Azarfar

Research Articles

Participatory epidemiology and associated risk factors of foot-and-mouth Disease in cattle in South Omo zone, South-Western Ethiopia 322
Bereket Molla, Gelagay Ayelet, Yilkal Asfaw, Yasmin Jibril and Esayas Gelaye

Study on prevalence of hydatidosis and cyst characterization in camels (Camelus dromedarius) slaughtered at Akaki abattoir, Ethiopia 329
Bulto Giro Boru, Yacob Hailu Tolossa, Getachew Tilahun and Hagos Ashenafi

Review

Probiotics in animal production: A review

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Accepted 8 July, 2013

Probiotics have been recently defined by the Food and Agriculture Organization/ World Health Organization (FAO/WHO) as live microorganisms which when administered in adequate amounts confer a health benefit on the host. A good probiotic should be non-pathogenic, non-toxic and capable of exerting beneficial effect on the host animal. It should be present as viable cells and capable of surviving and metabolizing in the gut environment. It should also be stable and capable of remaining viable for periods under storage and field conditions. Probiotics have been shown to promote growth, improve efficiency of feed utilization, protect host from intestinal infection and stimulate immune responses in farm animals. In laying chicken, probiotics increased hen-gay egg production. Weight gain performance was significantly increased in broilers and turkeys. In ruminants, probiotics also improved growth rate. Increased weight gain and higher efficiency of feed utilization were the results of the trials in pigs. Mortalities especially due to diarrhoea were reduced in pigs. The beneficial effects of probiotics in animal production have been related to different modes of action. The improvements in productive performance of all animal species fed with probiotics were mostly due to the fact that probiotics promoted the metabolic processes of digestion and nutrient utilization.

Key words: Probiotics, nutrient utilization, immune, animal production.

INTRODUCTION

Probiotics: Definitions

The term probiotic etymologically appears to be a composite of the Latin preposition *pro* ("for" or "in support") and the Greek adjective (biotic) from the noun *bios* ("life") meaning 'for life' or 'in support of life' and has had several different meanings over the years. It was first used by Lilley and Stillwell (1965) to describe substances secreted by one microorganism which stimulated the growth of another. It thus meant the exact opposite of antibiotic (Fuller, 1992). However, its use in this form did not persist and it was subsequently used by Sperti (1971) to describe tissue extracts which stimulated microbial growth. It was not until 1974 that Parker used it in the context in which we shall use it in this thesis. Parker defined probiotics as 'organisms and substances which

contribute to intestinal microbial balance (Parker, 1974). This definition related probiotic use to the intestinal microflora but the inclusion of substance gave it a wide connotation which would include antibiotics. In an attempt to improve the definition, Fuller (1989) redefined probiotics as 'a live microbial feed supplement which benefits the host animal by improving its intestinal microbial balance'. This revised definition stressed the need for a probiotic to be viable. Below is a chronicle of the evolution of definitions of probiotics:

1. Substances secreted by one microorganism that stimulate another microorganism (Lilly and Stillwell, 1965).
2. Tissue extracts that stimulate microbial growth (Sperti, 1971).

3. Organisms and substances that have a beneficial effect on the host animal by contributing to its intestinal microbial balance (Parker, 1974).
4. A live microbial feed supplement that beneficially affects the host animal by improving its intestinal microbial balance (Fuller, 1989).
5. A viable mono- or mixed culture of microorganisms that, applied to animals or humans, beneficially affects the host by improving the properties of the indigenous microflora (Havenaar, 1992).
5. A live microbial culture of cultured dairy product that beneficially influences the health and nutrition of the host (Salminen, 1996).
6. Viable bacteria, in a single or mixed culture, that has a beneficial effect on the health of the host (Donohue and Salminen, 1996).
7. Living microorganisms that on ingestion in certain numbers exert health benefits beyond inherent basic nutrition (Guarner and Schaafsma, 1998).
8. A microbial dietary adjuvant that beneficially affects the host physiology by modulating mucosal and systemic immunity, as well as improving nutritional and microbial balance in the intestinal tract (Naidu et al., 1999).
9. A preparation of or a product containing viable, defined microorganisms in sufficient numbers that alter the microflora (by implantation or colonization) in a compartment of the host and by that exert beneficial health effects in this host (Schrezenmeir and De Vrese, 2001).
10. Specific live or inactivated microbial cultures that have documented targets in reducing the risk of human disease or in their nutritional management (Isolauri et al., 2002).
11. Preparation of viable microorganisms that is consumed by humans or other animals with the aim of inducing beneficial effects by qualitatively or quantitatively influencing their gut microflora and/or modifying their immune status (Fuller, 2004).
12. Live microorganisms, which when administered in adequate amounts, confer a health benefit on the host (FAO/WHO, 2009).

HISTORICAL PERSPECTIVES OF PROBIOTICS

Although the use of the word probiotic in relation to feed supplements only dates from 1974, the history of live microbial feed supplements goes back thousands of years. Probably, the first foods that contained living microorganisms were the fermented milks that are recorded in the Old Testament (Genesis 18:8). There is also evidence from wall paintings dating back to 2500 B.C. that Sumarians were in the habit of inoculating milk to induce fermentation (Kroger et al., 1989).

Metchnikoff is regarded as the godfather of probiotic.

From his studies on probiotic and findings of his other coworkers, he wrote a book which in the original French edition published in 1907 was entitled "Essais optimistes". In the book, he discussed the philosophy, literature, religion, folklore and science of ageing. Only a small part of this discourse contained his views on the lower gut flora and the beneficial effects that fermented milk might have on it in humans. At the end of this section of the book, in the English edition, he concludes:

"If it be true that our precocious and unhappy old age is due to poisoning of the tissues (the greater part of the poisoning coming from the large intestine inhabited by numberless microbes), it is clear that agents which arrest intestinal putrefaction must at the same time postpone and ameliorate old age. This theoretical view is confirmed by the collection of facts regarding races which live chiefly on soured milk and amongst which great ages are common. However, in a question so important, the theory must be tested by direct observations. For this purpose, the numerous infirmaries for old people should be taken advantage of and systematic investigation should be made on the relation of intestinal microbes to precocious old age and on the influence of diets which prevent intestinal putrefaction in prolonging life and maintaining the forces of the body. It can only be in the future, near or remote, that we shall obtain exact information upon what is one of the chief problems of humanity".

In spite of these guarded statements, he is always quoted as having established a relationship between consumption of fermented milk and long life. This reputation was seemingly endorsed by the English translation of his book which was given the little "The Prolongation of Life". The consumption of fermented milk was given an added support by the publication in 1911 of a book by Londen Douglas called "The Bacillus of Long life". In the book, the author reiterated the connection between fermented milks and longevity. He also summarized what was known at that time of the Bacteriology of fermented milks (Douglas, 1911).

One of the most convincing demonstrations of the role of the gut microflora in resistance to diseases was provided by Collins and Carter (1978). They showed that the germ-free guinea-pig was killed by 10 cells of *Salmonella enteritidis* but it required 10^9 cells to kill a conventional grade animal with a complete gut microflora. There is thus, no doubt that animals have in their intestine a population of microorganisms that protects them against diseases. If that is the case, why do we need probiotics? Under normal conditions there would be no need for probiotics. In the wild, the young animal rapidly acquires a protective flora from its mother and the environment. However, modern methods of pre-natal care tend to limit the contact with the mother and provide

unnatural foods and unnatural environmental condition especially in poultry where after the egg is laid, the chick is permanently separated from their mother. The result is that the gut microflora is deficient in some of the normal components that are responsible for resistance to diseases.

Tannock (1983) reported that even the gut microflora of an adult can be affected by diet, antibacterial drugs and stress. The use of probiotic supplements seeks to repair these deficiencies. The development of probiotics has been in part stimulated by the public misgivings about the side effects that often follow the use of antibiotics as therapeutic agents and growth promoters. There is therefore a growing demand for an effective alternative to the antibiotic growth promoters, and probiotics could fill the gap. Recently, interest in the use of probiotics to improve the productive performance and general health status of livestock animals has been rekindled by legislations to curtail the use of sub – therapeutic doses of antibiotics in animal diets (Cook, 2000; Langhout, 2000; Mellor, 2000; Gill, 2001; Plail, 2006).

Characteristics of good probiotics

Fuller (1989) listed the following as features of a good probiotic:

1. It should be a strain, which is capable of exerting a beneficial effect on the host animal, for example increased growth or resistance to disease.
2. It should be non-pathogenic and non-toxic.
3. It should be present as viable cells, preferably in large numbers.
4. It should be capable of surviving and metabolizing in the gut environment for example, it should be resistant to low pH and organic acids.
5. It should be stable and capable of remaining viable for periods under storage and field conditions.

Benefits/advantages of probiotics

Intestinal probiotics, particularly bacteria play an important role in determining the digestive mechanisms and general health in all animals and humans (Fuller, 1992). The beneficial effects of probiotic will depend on a number of factors, including the strain chosen, level of consumption, duration and frequency of exposure, and the physiological condition of the individual (Koop-Hoolihan, 2001).

Some of the beneficial effects of the practical uses of probiotics are:

1. Growth promotion in farm animals (Mordenti, 1986; Chang *et al.*, 2001): Hydrocarbons are broken down by

probiotic bacteria which means the food is being split into its most basic elements. This allows almost total absorption through the digestive system. In this way, probiotics dramatically increase overall nutrition and enhance rapid cellular growth and development. For instance, *Lactobacillus* and *Bifidobacteria* increased weight gain and reduced mortality in young piglets (Abe *et al.*, 1995). Also, piglets fed *Bacillus coagulans* had lower mortality and improved weight gain and feed conversion than un-supplemented piglets and did as well as or better than piglets fed sub-therapeutic antibiotics (Adami and Cavazzoni, 1999).

2. Protection of host from intestinal infections (Nurmi and Rantala, 1973; Pascual *et al.*, 1999; Oyetayo *et al.*, 2003): The intestinal tract is cleansed by probiotics. They go under the layer of crud on the intestinal walls, attach themselves and dislodge the accumulated decay. This waste is then flushed out naturally. Yeast and fungal infections are prevented and sometimes eliminated with supplements of probiotics. Probiotic bacteria added to feed may protect piglets from intestinal pathogens by several possible mechanism, including competitive exclusion which entails adherence to intestinal mucosal thereby preventing attachment of pathogens, production of antimicrobial compounds (bacteriocins and organic acids), competition with pathogens for nutrients and stimulation of intestinal immune responses (Ellin, 2001).

3. Alleviation of lactose intolerance (Garvie *et al.*, 1984; Jiang, 1996): In humans, majority become lactase deficient during the 10 to 20 years of life and the inability to digest lactose causes a decrease in milk product consumption, eliminating a high quality source of protein and calcium. *Lactoba acidophilus* and *Lactoba bifidus* participate in the hydrolytic digestion of ingested lactose. Therefore, ingestion of milk product with live *Lactobacillus* is better tolerated and may actually alleviate malabsorption in lactose intolerant people (Fuller, 1992).

4. Relief of constipation (Graf, 1983): Constipation is quickly relieved by probiotics and the bowel movements become normalized. *Lactobacillus* can be taken both during and after antibiotic treatment. This helps in alleviating antibiotic-induced diarrhea caused by the indiscriminate killing off of both 'good' and 'bad' bacteria in the gastrointestinal tract (Fuller, 1992).

5. Anti-carcinogenic effect (Walker and Duffy, 1998; Zabala *et al.*, 2001). *Lactobacillus* inactivates carcinogenic intestinal beta-glucouronidase and nitroreductase. Studies at the Sloan Kettering Institute for Cancer Research and the University of Nebraska showed *Lactobacillus* to possess a definite anti-tumor activity and to inhibit tumor proliferation (Fuller, 1992). Animal studies have suggested that some lactic-acid bacteria might help protect against colon cancer, but more research is still needed.

6. Anticholesterolaemic effects (Tahri *et al.*, 1995):

Lactobacillus species possess anticholesterolemic and antilipidemic factors, which aid in cholesterol reduction. People that consume probiotics have experienced lowered cholesterol (Fuller, 1992).

7. Nutrient synthesis and bioavailability (Koop-Hoolihan, 2001): Probiotic bacteria synthesize certain amino acids, which are directly assimilated for example, lysine from specific strains of *L. plantarum*. They produce B vitamins, such as folic acid, niacin, riboflavin, B₁₂, B₆ and pantothenic acid, which are biocatalysts in food metabolism and help to fight stress (Fuller, 1992).

8. Probiotics has a protein-sparing effect: The lactobacillus primarily use carbohydrates as a growth medium, while the pathogens use primarily protein. By decreasing the pathogenic population, more protein is made available for assimilation (Fuller, 1992).

9. Prevention of genital and urinary tract infections (Redondo-Lopez et al., 1990; Martin et al., 1989): *Candida albicans* which is the primary yeast responsible for candidiasis has been shown to be inhibited by some probiotics (Fuller, 1992).

10. Immunostimulatory effects (Aattour et al., 2002): It has been discovered that conventional animals with a complete gut flora have increased phagocytic activity and immunoglobulin levels compared with germ-free animals. *Lactobacilli casei* in particular was found to be active in the stimulation of phagocytic activity when administered to mice (Perdigon et al., 1986).

Applications of probiosis to poultry

Probiotics for chicken are designed for two main reasons namely:

- (a) To replace beneficial organisms that is not present in the alimentary tract.
- (b) To provide the chicken with the effects of beneficial organisms.

Such beneficial organisms may be absent possibly because present methods of husbandry prevent contact between the newly hatched chicks and its parents preventing rapid vertical transfer of beneficial microorganisms or by management practices which may disturb intestinal microecology (Barrow, 1992). According to Barrow (1992), there are two major groups of probiotic preparations based on their site of action: those which are primarily intended to be effective in the crop and the anterior regions of the alimentary tract and those whose effects are directed at the caeca. However, it is likely that both types of preparation are to some extent, effective throughout the gut.

Among the first group are the lactobacillus cultures and preparations which are thought to colonize the crop and

small intestine in ways described by Fuller (1978). They are thought to exert antibacterial effects against potential pathogens (Fuller, 1974, 1978) and are also considered to increase performance by an unknown mechanism. There is less rationale for the later effect than the former. The assumption is that once pathogen burden is reduced, the animal will naturally perform better. Intestinal colonization is essential for the efficacy of probiotics. Whether many of these organisms actually become established in the gut is questionable since a number of criteria must be fulfilled to ensure colonization ability (Morishita et al., 1971; Fuller, 1978, 1986). These criteria may include adhesion to the crop epithelium, ability to grow in the nutritional environment of the gut and ability to resist innate or microbially produced inhibitory mechanisms.

From work with monocontaminated and dicontaminated gnotobiotic chicken, Morishita et al. (1971) found that whereas avian strains of *L. acidophilus*, *L. plantarum* and *L. fermentum* in addition to the non-intestinal *L. plantarum* and *L. casei* colonized well, a human *L. acidophilus* strain *L. melveticus* and *L. brevis* were rapidly eliminated from the alimentary tract. This indicated the importance of choosing both the right species and strain. A number of technical and experimental points must be considered in assessing the value of probiotic preparations and assessing experimental work carried out by others to do this.

Statistical and biological significance must be calculated but lack of significance in one area does not necessarily imply insignificance of the other (Barrow, 1992). Barrow further stated that statistical significance must be aimed for, but even if it cannot be attained the result may nevertheless be of biological significance. For example, a small but consistent weight gain may be economically significant for a large number of birds even if it is not statistically significance.

A critical review of the available literature on the application of probiosis to poultry performance and health is essential in assessing its value.

PROBIOTICS FOR CHICKEN

As with other mammals, the use of probiotics for poultry has developed out of our increasing understanding of the microflora of the gastrointestinal tract although an earlier observation suggested that the host and its intestinal micro flora were interdependent. This description of the intestinal microflora in adversarial terms was perpetuated by Dubos et al. (1965) who divided the indigenous microflora into the autochthonous organisms (such as *Lactobacilli* and *Bacteroides*, which had developed an evolutionary symbiotic relationship with the host) and allochthonous organisms (such as *Escherichia* and

Clostridium which were potential pathogens). These, together with non-enteric organisms acquired from the environment, comprised the normal intestinal flora. These descriptions are far too simplistic and must be seen as early models attempting to describe several highly complex ecosystems. For instance, microbial opportunism and true commensalisms were largely ignored. Regarding the flora as a climax community in which every niche is occupied is also patently inaccurate. Their inadequate understanding of microbial taxa at that time presumably led to regarding *Escherichia coli* as potential pathogen although many strains may be beneficial to the host and can be used in that way (Linton et al., 1978; Duval-Iflah et al., 1983). However, these hypotheses provided an important stimulus to studying the microecology of the alimentary tract. The early models had profound effect on the development of probiotics. Many preparations currently used for poultry and other animals are based on the assumption that the early hypotheses are correct with the result that the approach to probiosis is often too simplistic.

Probiotic effect on laying hens

A number of different cultures and products have been tested in laying hens producing equally variable results. Krueger et al. (1977) reported the results of feeding a so called Lactobacillus complex to young Leghorn hens at a concentration of 2.27 kg/ton. Three groups each of treated and control pens housing 26 young females and 2 males were monitored for 140 days. The treatment produced an improvement in egg production and feed efficiency of 3.03 and 7.41%, respectively. Crawford (1979) tested a mixed lactobacillus preparation at 340 g/ton in 101,615 commercial hens. The results showed an increase in egg production from 69.5% in control hens to 72.17% in treated birds. The amount of feed required to produce a dozen of eggs was reduced from 1.75 to 1.69 kg. Miles et al. (1981a) carried out a study at three sites: Florida, South Dakota and Arizona. A mixed lactobacillus was again tested in the feed at varied levels of 0.0125, 0.0375 and 0.0625%. The viable counts of different batches of probiotic were estimated at a minimum of 4×10^6 organisms per gram. The treated and untreated feed was given to seven groups of ten layers from 24 weeks of age for 280 days. The results revealed an increase in egg production in Florida with concentrations of 72.77, 72.57 and 70.88% in treated birds compared with 70.89% for the control. Similar results were obtained at Arizona but not at South Dakota. The absence of an increase at the higher level was attributed to excessive numbers of organism, but this again suggest that probiotic is not dose dependent rather it is threshold dependent (Numan, 2001).

Yoruk et al. (2004) reported that supplementation of layers' diet humate and probiotic resulted in increase in egg production and a decrease in mortality. They also observed that the treatment did not have any effect on egg quality. Similarly, Ezema (2012) observed that supplementation of layers' diets with varied levels of probiotic (*Saccharomyces cerevisiae*) significantly increased ($p < 0.05$) hen-day egg performance but did not have any significant effect ($p > 0.05$) on egg quality.

Effects on broiler performance

Couch (1978) reported several studies in broilers. In the first study, a lactobacillus strain was incorporated in the feed at 0.025, 0.0375, 0.05, 0.0625 and 0.075%. The birds were stressed due to abnormal cold weather. The results showed an increase in growth rate in males of 7 to 10% and in the females of 5 to 6% among all the treated groups. In a second study, chicken in battery cages were given feed containing 0.05, 0.1 and 0.2% cultures for a period of 3 weeks. The diet had suboptimal levels of amino acid. But there was an accelerated growth when lactobacillus was administered. Couch suggested, in line with many other claims that probiotics are of particular use when poultry are reared under stressful conditions. It is important to add here that the poultry production system in the tropics is under serious stress due to high ambient temperature and other management inadequacies.

Avends (1981) administered a bile acid-resistant Lactobacillus strain via the drinking water to broilers held under field conditions. In the first trial, four houses of birds containing 116,000 broilers were given 10^6 lactobacilli per day for 30 days. The controls consisted of two houses of 58,242 birds. A 6% weight increase and 3% feed conversion increase were observed. In a second trial comprising 31,000 birds in treated and control groups, a 3% weight increase and 1% feed conversion increase was obtained.

In another study, Ezema (2007) used a total of 140 day-old broiler chicks (Anak, 2000) which were randomly divided into seven groups of 20 birds each. Each group was subdivided into four replicates of five birds each. Groups 1 to 5 were placed on experimental diet made of 70% basal diet and 30% PKC. Groups 1 to 4 had probiotic supplement at varied levels of 0.4, 0.8, 1.2 and 1.6 gm yeast/kg of feed, respectively. Group 5 had no yeast (control 1). Groups 6 and 7 had no PKC (normal broiler diet) but group 6 had 1.2 gm yeast/kg of feed. Group 7 had no yeast (control 2). Group 2 weighed significantly heavier ($p < 0.05$) than the rest. Groups 2 and 3 had the highest apparent crude fibre digestibility of 30.86 and 30.87%, respectively. The cost of feed to produce 1 kg live weight gain of group 2 was ₦129.85 ±

2.17/kg, group 5 was ₦154.00 ± 2.08/kg and group 7 was ₦192.28 ± 6.84/kg. Group 2 performed significantly better than others in weight gain, carcass weight and economic gain. Based on the results of this study, 0.8 gm yeast/kg of feed was recommended for optimum broiler production in the tropics.

In a recent study in breeding layer and broiler hens using different probiotic preparations, Bozkurt et al. (2011) observed that egg production rate, egg weight and egg mass benefited from some of the probiotics while overall, the probiotics led to significant improvement in the feed conversion ratio of layer hens. These research workers further reported that no study has yet shown that probiotic feeding has any detrimental effects on health status and productivity.

Probiotic effects on turkeys

Probiotic cultures have also been administered to turkeys and other poultry. In several separate studies, the mixed lactobacillus preparation described in broilers has been assessed in turkeys (Dilworth and Day, 1978). Francis et al. (1978) tested the commercial preparation in groups of 48 broad-breasted large white turkey poulters administering 750 mg per kg in the feed for 3 weeks. An increase in body weight from 4.11.8 to 424.6 g was observed but feed efficiency fell slightly from 1.40 to 1.39.

This probiotic was also tested by Crawford (1979) who found a 6.1% weight increase at 12 weeks of age after continuous administration of 0.2 kg per ton. In a study using 72 (15 days old) white hybrid converter turkey poulters, Cetin et al. (2005) investigated the effects of manna oligosaccharide (MOS) and probiotic supplementation on haematological and immunological parameters of turkeys. The experiment showed that probiotic supplementation caused significant increase ($p < 0.05$) in the erythrocyte count, haemoglobin concentration and haematocrit values, but MOS supplementation did not have any significant effect ($p > 0.05$) on these parameters. This study also revealed that both the probiotic and MOS supplementation resulted in significant increases ($p < 0.05$) in the serum IgG and IgM levels. This trial suggests that MOS and probiotic that enhanced immunoglobulin levels will have more positive effects on growth performance and turkeys' ability to resist diseases.

It has been demonstrated that direct fed microbial (DFM) may offer an effective alternative to antibiotic growth promoter in turkeys. Wolfenden et al. (2011) identified 4 *Bacillus* isolates and evaluated their potential as DFM candidates. These isolates were shown to significantly increase body weight gain as well as reduce recovery of *Salmonella* after experimental infection.

Higgins et al. (2005) investigated the effects of selected

probiotic bacteria on performance of poulters in 3 separate commercial turkey brooder houses. In all the experiments, treatment of probiotic cultures or antibiotics were administered in water. Poulters were tagged and placed into individual pens (20 per pen, 4 replicates per treatment). Performance was evaluated by body weight or body weight gain. In the first experiment poulters received 1 of 2 probiotic cultures and weighed significantly more than non-treated or antibiotic treated poulters. In the second experiment, there was no significant difference among any of the groups. In the third experiment which was performed during clinically significant *Salmonella seftenburg* infection, poulters that received antibiotic followed by a probiotic culture had significantly higher weight gain than non-treated poulters.

In another study, Torres-Rodriguez et al. (2007) evaluated the effects of probiotic culture in combination with dietary lactose as a prebiotic in two experiments. Treated poulters (*Lactobacillus* spp. based probiotic culture) received dietary lactose (0.1%) continuously in the feed and probiotic culture (~10 cfu/ml) in the drinking water. Three hundred and twenty selected female poulters were tagged and randomly divided into 2 treatments with 4 replicates each ($n = 40$). The poulters in experiment 1 were challenged with $\sim 10^4$ cfu of *Salmonella enteritidis* but experiment 2 was not challenged. Body weights were determined on days 1, 7 and 14 (experiment 1 trial 1 and 2, experiment 2 trial 3) and on day 1, 8 and 18 (experiment 2 trial 4). Body weight and Feed Conversion Ratio (FCR) were significantly ($p < 0.05$) improved by treatment in *Salmonella* challenged poulters (trials 1 and 2). In contrast, unchallenged turkey poulters (trials 3 and 4) showed no significant difference ($p > 0.05$) in either body weight or FCR. These results suggest that dietary lactose with appropriate probiotic organisms may enhance performance of poulters following a mild pathogenic challenge.

Vicente et al. (2007) studied the ability of 2 probiotic cultures (P1 and P2) to reduce conventional *Salmonella* in commercial turkey flocks 2 weeks prior to processing with or without the use of a commercial organic acid. Two weeks after treatment, the recovery of *Salmonella* was significantly reduced ($p < 0.05$) in houses in which P1 and P2 cultures were administered in combination with organic acid. The results indicate that administration of selected probiotic candidate bacteria in combination with organic acid, may reduce environmental *Salmonella* in turkey houses prior to live haul and that this practise could help to reduce the risk of *Salmonella* in the processing plant.

Probiotic effects on ruminants

Yeast and yeast-containing products has been used in

ruminants' diets for many years as a source of protein and energy (Eckles and Williams, 1925; Carter and Philips, 1944). However, since the late 1980s there has been an enormous increase in interest in products based on yeast and/or filamentous fungi that are analogous to probiotics and which enhance gut functions (Wallace and Newbold, 1992). These researchers stated that products based on yeast or fungi were fed to adult ruminants to achieve a production response that is unrelated to the prevention of diarrhea. They further observed that these products nevertheless improved the nutrition of growing or adult ruminants much more than would be expected from their gross nutrient content. The products in current use contain either the yeast (*Sac. Cerevisiae*) or the aerobic fungus (*A. oryzae*) or sometimes both together, hence they are described loosely as fungal feed additives or fungal probiotics. The effectiveness of fungal probiotics stem from their influence on rumen fermentation, so they fall into the category of rumenal modifiers among the so called growth promoters (Wallace and Newbold, 1992).

The effects of a commercial preparation called "yeasture" which composed of live yeast cultures from three strains of *Saccharomyces cerevisiae* in combination with probiotic bacteria and enzymes were investigated on 80 Holstein – Friesian cows divided into two groups (Sretenovic et al., 2008). The diets for the two groups were identical and the trial group received 10 g of "yeasture" daily. The application of "yeasture" started 15 days prior to calving and lasted until 60th day of lactation. The study showed that the commercial preparation (yeasture) influenced quantity and composition of the milk. The difference between the trial and control groups was 2.57 kg 4% FCM or 8.70% ($p < 0.01$) and 7.16% milk fat ($p < 0.05$). Supplemented group had lower somatic cells count by 7.3% which indicated better health of the cows' udder.

Probiotic effects on pigs

In pigs, several organisms are used as probiotics while others are under investigation as potential probiotics (Tuschy, 1986; Tournut, 1989). The major aim of using probiotics in pigs is to improve the performance and health of the animals. Growth rate, efficiency of feed utilization, mortality and number of days with diarrhea, sometimes irrespective of the cause, are most commonly measured (Jonsson and Conway, 1992).

Wilcock (2011) reported that supplementing live yeast in pigs' diet during lactation increased the quantity and quality of the milk produced and improve the growth rate (+ 12%) of piglets. The researcher further observed that weaning weight was increased by 0.73 kg while increasing number of pigs weaned by 0.42 pigs per litter.

CONCLUSION

The beneficial effects of probiotics in animal production have been related to different modes of action. The improvement in productive performance of all poultry species fed with probiotics were mostly due to the fact that probiotics promoted the metabolic processes of digestion and nutrient utilization. Experimental studies have shown that probiotic dietary supplementation might influence these mechanisms by exerting enzymatic activities, increasing the passage rate of digestion and deconjugating bile salts and acids. It is believed that the improvement in metabolic processes that were observed as a result of probiotic supplementation were due to improved development of the gut and increased microvilli height which led to the enlargement of the microvilli's absorptive surface and enabled the optimal utilization of nutrients.

REFERENCES

- Aattour N, Bouras M, Tome D, Marcos A, Lemonnier D (2002). Oral ingestion of Lactic acid bacteria by rats increases lymphocyte proliferation and interferon production. *Br. J.* 87:367-373.
- Abe F, Ishibashi N, Shimamura S (1995). Effect of administration of *Bifidobacteria* and lactic acid bacteria to newborn calves and piglets. *J. Dairy Sci.* 78 (912):2838-2846.
- Adami A, Cavazzoni V (1999). Occurrence of selected bacterial groups in the faeces of piglet feed with *Bacillus coagulans* as probiotic. *J. Basic Microbiol.* 39 (1):3-9.
- Avends` LG (1981). Influence of *L. acidophilus* administer via the drinking water on performance of broilers. *Poult. Sci.* 60:1617 (Abstract).
- Barrow P A (1992). Probiotics for chicken (in *Probiotics: The scientific basis*, ed. by R. Fuller). Chapman and Hall, London. pp 225-257.
- Carter HE, Philips GE (1994) The nutritive value of yeast proteins. *Fed. Proc.* 3, 123-8.
- Cetin N, Guclu BK, Cetin E (2005). The effects of probiotic and mannanoligosaccharide on some haematological parameters in turkeys. *J. Vet. Med. Physio. Pathol. Clin. Med.* 52(6):263-7.
- Chang Y, Kim J, Kim W, Kim Y, Park Y (2001). Selection of a potential probiotic *Lactobacillus* strain and subsequent *in-vivo* studies. *Antonne Van Leeuwenhoek*. pp. 193-199.
- Collins FM, Carte PB (1978). Growth of salmonellae in orally infected germfree mice. *Infect. Immunol.* 21, 41-7.
- Cook M (2000). Alternatives to antibiotics found for growing chickens. *World Poult.* 16: 45.
- Couch JR (1978). Poultry researchers outline benefits of bacteria, fungistatic compounds, other feed additives. *Feedstuffs*, 50, 6.
- Crawford JS (1979). 'Probiotics in animal nutrition. *Proc. 1979 Arkansas Nutr. Conf.* 45-55.
- Dilworth BC, Day EJ (1978). *Lactobacillus* cultures in broiler diets. *Poult. Sci.* 57, 1101 (Abstract).
- Donohue DC, Salminen S (1996). Safety of probiotic bacteria. *Asia Pac. J. Clin. Nutr.* 5:25–8.
- Duval-Iflah Y, Chappuis JP, Ducluzea R, Raibaud P (1983). Intra-specific interactions between *Escherichia coli* in human newborns and in gnotobiotic mice and piglets. *Progress in Food and Nutrition Science.*7;107-16.
- Eckles CH, Williams VM (1925). Yeast as a supplementary feed for lactating cows. *J. Dairy Sci.* 8, 89-93.
- Ellin DM (2001). Alternatives to Antibiotic use of growth promotion in animal husbandry. Food Research Institute Briefings.

- Ezema C (2007). The performance of broilers fed palm kernel cake-based diet supplemented with bioactive yeast. M.Sc Dissertation. Dept. of Animal Health and Production, University of Nigeria, Nsukka. p.47.
- Ezema C (2012). Probiotic Effects of *Saccharomyces cerevisiae* on Laying Chicken Fed Palm Kernel Cake-Based Diets. PhD Thesis, Department of Animal Health and Production, Faculty of Veterinary Medicine, University of Nigeria, Nsukka. p 84.
- FAO (2009) Food and Agriculture Organization of the United Nations. Guidelines for the evaluation of probiotics in food. Available at: <ftp://ftp.fao.org/es/esn/food/wgreport2.pdf> (accessed January 27, 2009).
- Francis C, Janky DM, Arafaharms RH (1978) Interrelationship of lactobacillus and zinc bacteria in the diets of turkey poult. *Poult. Sci.* 57, 1687-9.
- Fuller R (1974). Ecological studies on the lactobacillus flora associated with the crop epithelium of the fowl. *J. Appl. Bacterio.* 36, 131-9.
- Fuller R (1986). Probiotics. *Journal of Applied Bacteriology Symposium.* No. 15, Pp. 1-7.
- Fuller R (1989). Probiotics in man and animals. *J. Appl. Bacteriol.* 66, 365-378.
- Fuller R (1992). Probiotics: the Scientific basis. Ed. Fuller R London; Chapman and Hall.
- Fuller R (2004). What is a probiotic? *Biologist* , 51:232.
- Fuller R. (1978). Epithelial attachment and other factors controlling the colonization of the intestine of the gnotobiotic chicken by *Lactobacilli*. *J. Appl. Bacteriol.* 4B:335-342.
- Garvie EI, Cole CB, Fuller R, Hewitt D (1984). The effect of yoghurt on some components of the gut micro flora and the metabolism of lactose in the rat. *J. Appl. Microbiol.* 56:237-245.
- Gill C (2001). Safe and sustainable feed ingredients. *Feed International.* 22:40-45.
- Graf W (1983). Studies on the therapeutic properties of acidophilus milk. *Symposia of Swedish Nutrition Foundation XV*, 119-121.
- Guarner F, Schaafsma GJ (1998). Probiotics. *Int. J. Food Microbiol.* 39:237-8.
- Havenaar R (1992). *Huis In't Veld JMJ. Probiotics: a general view.* In: Wood BJB, ed. *Lactic acid bacteria in health and disease.* Vol 1. London: ElsevierApplied Science Publishers; p. 151-70.
- Higgins SE, Torres-Rodriguez A, Vicente JL, Sartor CD, Pixley CM, Nava GM, Tellez G, Barton JT, Hargis BM (2005). Evaluation of Intervention Strategies for Idiopathic Diarrhea in Commercial Turkey Brooding Houses. *J. Appl. Poult. Res.* 14:345-348.
- Isolaure E, Rautava S, Kalliomaki M (2002). Role of pro-biotics in food hypersensitivity. *Current Opinion in Immunological Clinical Allergy*, 2:263-271.
- Jiang T, Mustapha A, Savaiano DA (1996). Improvement of lactose digestion in humans by ingestion of unfermented milk containing *Bifidobacterium longum*. *J. Dairy Sci.* 79 (5):750-757.
- Jonsson E, Conway P (1992). Probiotics for pigs (in *Probiotics: The scientific basis.* Ed. by R. Fuller) Chapman and Hall, London. pp 259-316.
- Koop-Hoolihan L (2001). Prophylactic and therapeutic uses of Probiotics: *A Review of Journal of the American Dietetic Association.* 147:747-748.
- Kroger M, Kummann JA, Raic JL (1989). Fermented milks – past and present. *Food Technology.* 43, 92-9.
- Krueger WF, Bradley JW, Patterson RH (1977). The interaction of gentian violet and lactobacillus organisms in the diet of Leghorn hens. *Poult. Sci.* 56, 1729 (Abstract).
- Langhout P (2000). New additives for broiler chickens. *World Poultry.* 7: 18-19.
- Lilly DM, Stillwell RH (1965). Probiotics: Growth promoting factors produced by microorganisms. *Science.* 147:747-748.
- Linton AH, Howe K, Richmond MH (1978). Attempts to displace the indigenous antibiotic resistant gut flora of chickens by feeding sensitive strains of *Escherichia coli* prior to slaughter. *J. Appl. Bacteriol.* 45, 221-227.
- Martin SA, Nisbel BJ, Dean RF (1989). Influence of a commercial yeast supplement on the in-vitro ruminal fermentation. *Nutr Reprod. Int.*
- Mellor S (2000). Nutraceuticals – alternatives to antibiotics. *World Poultry.* 16:30-33.
- Metchnikoff E (1907). The prolongation of life. *Optimistic studies.* G.P. Putman, sons. Mitswka T (1992). The human gastrointestinal tract. In the lactic acid bacteria in health and disease Vol.I. ed Wood B.J. D. Elsevier Appl. Sci. London. 69-114.
- Miles RD, Arafa AS, Harms RH (1981a). Effects of a living non-freeze dried lactobacillus culture on performance, egg quality and gut micro flora in commercial layers. *Poult. Sci.* 60, 993-1004.
- Morishita Y, Mitsuoka T, Kaneuchi C (1971). Specific establishment of lactobacilli in the digestive tract of germfree chickens. *Japan J. Microbiol.* 15, 531-8.
- Naidu AS, Bidlack WR, Clemens RA (1999). Probiotic spectra of lactic acid bacteria (LAB). *Crit. Rev. Food Sci. Nutr.* 39:13-126.
- Numan O (2001). Heterologous expression of genes in the yeast *Saccharomyces cerevisiae* Turk, *J. Agric.* 25: 45-49.
- Nurmi E, Rantala M (1973). New aspects of *Salmonella* infection in broiler production. *Nature.* P. 241, 210-211.
- Oyetayo VO, Adetuyi FC, Akinyosoye FA (2003). Safety and protective effect of *Lactobacillus acidophilus* and *Lactobacillus casei* used as a probiotic agent *in vivo*. *Afr. J. Biotechnol.* 2 (11):448-452.
- Pascual M, Hugas M, Badiola JI, Monfort JM, Garriga M (1999). *Lactobacillus salivarius* CTC 2197 prevents *Salmonella enteritidis* colonization in chicken. *Appl. Environ. Microbiol.* 65: 4981-4986.
- Perdigon G, Macais MEN, Alvarez S, Oliver G, De Ruiz-Holgado AAP (1986). Effect of perorally administered *Lactobacilli* on macrophage activation in mice. *Infect. Immun.* 53: 404-410.
- Plail R (2006). The innovative power of probiotics. *Poult. Int.* 45: 34- 36.
- Redondo-Lopez V, Cook RL, Sobel JD (1990). Emerging role of *Lactobacilli* in the control and maintenance of the vaginal bacterial microflora *Rev. Infect. Dis.* 12:856-872.
- Salminen S (1996). Uniqueness of probiotic strains. *International Dairy Feeding Sand Nutrition Newsletter.* 5:16-18.
- Sperti GS (1971). Probiotics. West Point (CT): AVI Publishing Co.; 1971.
- Tahri K, Crociani J, Ballongue J, Schneider F (1995). Effects of three strains of *Bifidobacteria* on cholesterol. *Lett. Appl. Microbiol.* 21(3):149-151.
- Tannock GW (1983). Effects of dietary and environmental stress on the gastrointestinal microbiota, in *Human Intestinal Microflora in Health and in Disease*, (ed. Hentges DJ) Academic Press, New York. pp. 517-39.
- Torres-Rodriguez A, Higgins S., J. Vicente, Wolfenden, A., Gaona-Ramirez, G., Barton, J., A. M. Donoghue, G. Tellez, B. M. Hargis (2007) Effect of Lactose as a Prebiotic on Turkey Body Weight Under Commercial Conditions. *J. Appl. Poult. Res.* 16:635-641.
- Tournut J (1989). Applications of probiotics to animal husbandry. *Review of Science and Technology Office of International Epizootics.*, 8, 553 - 566.
- Tuschy D (1986). Verwendung von Probiotika als Leistungsförderer in der Tierernährung, Übers. *Tierenährg*, 14, 157-178.
- Vicente J S, Higgins LB, Tellez G., Donoghue A, Hargis BM (2007). Effect of Probiotic Culture Candidates on Salmonella Prevalence in Commercial Turkey Houses. *J. Appl. Poult. Res.*:16:471-476.
- Walker AW, Duffy LC (1998). Diet and bacterial colonization: Role of probiotics and prebiotics: *Rev J. Nutr. Biochem.* 9:668-675.
- Wallace RJ, Newbold CJ (1992). Probiotics for ruminants (in *Probiotics: The scientific basis.* Ed. by Fuller R) Chapman and Hall, London. pp 317-353.
- Wilcock P (2011). Piglets performing better with yeast during lactation. *Pig Progress*, Vol. 27, No. 3. pp 22-23.
- Wolfenden RE, Pumford MJ, Morgan S, Shivaramaiah AD, Wolfenden CM, Pixley J, Green G, Tellez G, Hargis BM (2011). Evaluation of selected direct-fed microbial candidates on live performance and Salmonella reduction in commercial turkey brooding houses. *Poult. Sci.* 90:2627-2631.

Zabala A, Martin MR, Haza AI, Fernandez L, Rodriguez JM, Morales P (2001). Anti-proliferative effect of two lactic acid bacteria strains of human origin on the growth of a myeloma cell line. *Lett. Appl. Microbiol.* 32:287-292.

Review

A review of the role of five kinds of alternatives to in-feed antibiotics in broiler production

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Accepted 2 October, 2013

In view of severe restriction of total ban on the use of antibiotics as growth promoters and therapeutic agents in poultry industry, the search for alternatives to replace antibiotics has gained increasing interest in animal nutrition. Gut micro flora appears to be the target for IFAs and alternatives to exert health benefits and some growth-promoting effects. Subsequent to banning of use of antibiotics as growth promoter in poultry nutrition, numerous studies turned to finding of alternative solutions, that is, other natural substances, which would have positive effect on chicken growth and feed conversion. Today, several groups of these additives are in use and most often probiotics, prebiotics, synbiotic, acidifiers and phytobiotics additives. Considering that each of the stated groups has its own specificities, the objective of this work was to present main mechanism of their action and to present their effect on production results in fattening of broiler chickens through review of research published in this field.

Key words: Broilers, probiotics, prebiotics, phytobiotics, synbiotic, acidifiers

INTRODUCTION

Growth promoters are chemical and biological substances which are added to livestock food with the aim to improve the growth of chickens in fattening, improve the utilization of food and in this way realize better production and financial results. Their mechanism of action varies. Positive effect can be expressed through better appetite, improved feed conversion, stimulation of the immune system and increased vitality, regulation of the intestinal micro-flora, etc. A probiotic is a live microbial feed supplement, which beneficially affects the host animal by improving its intestinal balance. It has been used as a substitute of antibiotics that is being used in considerable amounts as growth promoters in broilers production and is, associated with incalculable risks for human health resulting from the use of particular feed additives. Probiotics are one of the approaches that have a potential to reduce chances of infections in poultry and

subsequent contamination of poultry products (Bellisle et al., 1998). Prebiotics are selectively fermented, dietary ingredients that result in specific changes in the composition and/or activity of the gastrointestinal microbiota, thus conferring benefit(s) upon host health (Zhan et al., 2003). Aromatic plants (phytobiotics) have been used since ancient times for their preservative and medicinal properties and to impart aroma and flavor to food. Hippocrates, the 'father of medicine', used plant extracts and prescribed perfume fumigations. For centuries, aromatic plants, also known as herbs and spices, their essential oils and herbal extracts have been used as natural pharmaceuticals in traditional medicine and veterinary medicine. However, their use has not been based on rigorous scientific investigation, but has stemmed from ethno veterinary or even folkloric sources (Chang, 2000). The ban on the use of antimicrobial growth promoters

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within the EU (Barton, 1999) and the demand by consumers for safe products has renewed the interest in aromatic plants and their extracts mainly as a source of alternative therapeutics or natural antioxidants. Synbiotics is defined as a mixture of probiotics and prebiotics that beneficially affects the host by activating the metabolism of one or a limited number of health promoting bacteria and by selectively stimulating their growth, hence improving the hosts welfare (Collins and Gibson, 1999). Recent research showed that synbiotic products improved immune status in broiler chicks (Zhang et al., 2006). Organic acids are mixed with the feed to create an acidified pH which provides a favorable environment in the digestive tract of broilers for the effective digestion of dietary nutrients such as proteins. They act as growth promoters and feed preservatives in poultry where they can also maintain feed hygiene. Also organic acids improve protein and energy digestibility by reducing the microbial competition nutrients of the host, endogenous nitrogen losses and ammonia production are other beneficial effects for broilers (Dibner and Buttin, 2002). In any case, expected results of the use of these additives are increased financial effects of production. Because of the fact that growth promoters have different mechanisms of action, it is necessary to present every group individually and present the effect which can be expected with their utilization (Nahashon et al., 1996; Jin et al., 1998; Fuller, 1997).

PROBIOTICS

Probiotics are organisms and substances which help to improve the environment of the intestinal tract (Green and Sainbury, 2001). Certain species of bacteria, fungi and yeasts belong to group of probiotics. Existing probiotics can be classified into colonizing species (*Lactobacillus* sp., *Enterococcus* sp. and *Streptococcus* sp.) and free, non colonizing species (*Bacillus* and *Saccharomyces cerevisiae*) (Zikic et al., 2006). Lilley and Stillwell (1965) first introduced the term "Probiotic" to describe, "growth promoting factors" produced by microorganisms. The word "probiotic" is derived from the Greek word 'probios' meaning 'for life' and has had several different meanings over the years. Parker (1974) used the term probiotics for microorganisms or substances that contribute to intestinal microbial balance. Fuller (1989) redefined the probiotic as "A live microbial feed supplement, which beneficially affects the host animal by improving the intestinal microbial balance". As mentioned by Fuller (1992) and Anonymous (2002), several microorganisms have been used as probiotics, containing bacteria belonging to genus *Bifidobacterium*; bacteria belonging to genus *Lactobacillus*; bacteria belonging to genus *Streptococcus*; yeast belonging to genus *Saccharomyces*; yeast belonging to genus *Candida*; Moulds; *Bacillus subtilis* etc.

Probiotics display several ways of action: Antagonistic

action towards pathogen bacteria by secretion of products which inhibit their development, such as bacteriocins, organic acids and hydrogen peroxide; the other way is competitive exclusion which represents competition for locations to adhere to the intestinal mucous membranes and in this way pathogen microorganisms are prevented from inhabiting the digestive tract, and the third way is competition for nutritious substances (Patterson and Brukholder, 2003). Vranesic (1992) reviewed the use of probiotics, live bacterial and or fungal cultures, as feed supplements and concluded that the probiotics stimulated numerous metabolic processes relating to feed digestion and absorption. It was also opined that few authors also include enzymes, yeasts and even organic acids in the group of probiotics. Hennig et al. (1993) evaluated the use of probiotics as growth promoters and opined that the experiments when supplemented with probiotics must end at a given weight. Palod and Singh (2004) indicated that the 'Probiotics' in broiler feeding was becoming a new area in biotechnology and offer a possible replacement for the use of sub-therapeutic level of antibiotics in broiler feeds. The probiotics include more than 200 species of bacteria and yeast. The various probiotics available in the market are either single or combination of bacteria, yeast and fungi. The use of probiotics in broiler feed causes better growth, higher feed conversion, better digestibility and improved product quality. The other results showed that adding primalc probiotics caused a decrease in the blood cholesterol, blood uric Acid and blood urea (Rezaei et al., 2013). Georgieva et al. (2000) observed a significant weight gain by less feed consumption at 49 days of age in broiler chicken when supplemented with a commercial probiotics, Lacto-Sacc compared to controls and antibiotic treated groups. Bhat et al. (2003) reported that the probiotic mixture containing *Lactobacillus sporogenes* 30,000 million cfu., *Lactobacillus acidophilus* 30,000 million cfu., *Sac. cerevisiae* SC – 47 1,25,000 million cfu., Alpha amylase 5 gm and sea weed extract 50 gm/kg when fed to broiler chicken at the rate of 0.1% in feed improved the body weight gain, feed consumption and feed conversion ratio. Panda et al. (2000) reported probiotic had no influence on dressing percentage or weight of internal organs such as liver, heart and gizzard. According to Mandal et al. (1994) there was no significant increase in body weight gain in Bioboost®, a commercial probiotic containing *Sac. cerevisiae* and *Bacillus coagulans* (*L. sporogenes*), supplemented group. They also reported serum biochemical components such as serum protein (5.70 ± 0.50 g/100 mL), serum calcium (9.00 ± 0.42 mg per 100 ml) and serum phosphorus (7.20 ± 0.42 mg/100 mL) which did not differ significantly between control and probiotics supplemented groups.

PREBIOTICS

Gibson and Roberfroid (1995) defined a prebiotic as a

non-digestible food ingredient which beneficially affects the host by selectively stimulating the growth of and/or activating the metabolism of one or a limited number of health-promoting bacteria in the intestinal tract, thus improving the host's microbial balance. The growth of endogenous microbial population groups such as bifidobacteria and lactobacilli is specifically stimulated and these bacteria species are perceived as beneficial to animal health. Fructo-oligosaccharides were shown to support the growth of beneficial bacteria, such as lactobacilli (Xu et al., 2003; Yusrizal and Chen, 2003; Zhan et al., 2003), but failed to stimulate the growth of bifidobacteria (Vidanarachchi et al., 2006). It is reported that rapid fermentation of prebiotics, leading to high concentrations of organic acids, impaired the barrier function, which reduced the ability of rats to resist salmonella infection (Ten Bruggencate et al., 2003). It may also be worthwhile to examine the interaction between prebiotics(s) and bird sex. In the report by Yusrizal and Chen (2003), body weight and feed conversion ratio (FCR) of female birds were improved by 10 and 9% respectively, on oligofructose treatment but no such effects were observed in males. Similar results were stated also by Mateo et al. (2000). This proves that effect of application of prebiotics depends on the condition of animals, environment conditions, composition of food and level and type of prebiotic included in the mixtures. Maiorka et al. (2001) conducted an experiment with the treatments such as T1-no additives, T2-antibiotics (Olaquinox and Nitrovin), T3- prebiotic (0.2% *Sac. cerevisiae* cell wall), T4-probiotic (300 ppm *Bacillus subtilis*) and T5-symbiotic (T3+T4) and observed better live weight gain in broilers up to 45 days of age, fed with symbiotics followed by antibiotics, prebiotics and probiotics. The total absence of additives in the diets worsened broiler chicken performance. Prebiotics have been shown to alter gastro-intestinal micro flora, alter the immune system, prevent colonic cancer, reduce pathogen invasion including pathogens such as *Salmonella enteritidis* and *Escherichia coli* and reduce cholesterol (Cummins and Macfarlane, 2002). The other results indicated that addition of prebiotic supplementations to broiler diets, improved growth performance, carcass characteristics and decreased serum cholesterol level of the broiler chickens at 42 day of age (Fallah and Rezaei, 2013).

SYNBIOTICS

A synbiotic is, in its simplest definition, a combination of probiotics and prebiotics (Collins and Gibson, 1999). This combination could improve the survival of the probiotic organism, because its specific substrate is available for fermentation. This could result as an advantage to the host through the availability of the live microorganism and the prebiotic. Recent research showed that symbiotic products improved immune status in broiler chicks

(Zhang et al., 2006). According to (Awad et al., 2008) an investigation, synbiotics can lead to better absorption of glucose in poultry. Synbiotic product had a comparable potential to improve broiler performance as avilamycin (an antibiotic growth promoter) (Mohnl et al., 2007). Liang and Shah (2006) concluded that the use of synbiotics consumption in broilers regulates the concentration of the organic acids and reduce cholesterol levels. Bailey et al. (1991) used a combination of Fructooligosaccharides and competitive exclusion flora to reduce *Salmonella* colonization in chickens. The combination was more effective in reducing *Salmonella* colonization Fructooligosaccharides than or in competitive probiotic alone.

PHYTOBIOTICS

Plant products have been used for centuries by humans as food and to treat ailments. Natural medicinal products originating from herbs and spices have also been used as feed additives for farm animals in ancient cultures for the same length of time. To differentiate from the plant products used for veterinary purposes (prophylaxis and therapy of diagnosed health problems), phytobiotics were redefined by Windisch and Kroismayr (2006) as plant-derived products added to the feed in order to improve performance of agricultural livestock. Mechanism of the action of these additives is not completely clear. Some plant extracts influence digestion and secretion of digestive enzymes and besides, they exhibit antibacterial, antiviral and antioxidant (Cross et al., 2007). Antimicrobial activity and immune enhancement probably are the two major mechanisms by which phytobiotics exert positive effects on the growth performance and health of animals. Phytochemicals in phytobiotics are well known to have antimicrobial ability (Cowan, 1999). Polysaccharide components are considered to be the most important immune active components (Xue and Meng, 1996). A common feature of phytobiotics is that they are a very complex mixture of bioactive components. For example, hawthorn fruit, a common growth-enhancing and digestion modifier, has been shown to contain more than 70 kinds of organic chemicals along with some unidentified factors and active bio-active compounds (Wang et al., 1998). Therefore they may exert multiple functions in the animal body. Increased feed intake and digestive secretions are also observed in animals offered phytobiotic-supplemented feed (Windisch and Kroismayr, 2006). Growth enhancement through the use of phytobiotics is probably the result of the synergistic effects among complex active molecules existing in phytobiotics (Gauthier, 2002). However, the exact growth promoting mechanisms of phytobiotics in broiler chickens are poorly understood. Four factors may affect the effectiveness of phytobiotic additives: plant parts and their physical properties; source; harvest time and compatibility with the other ingredients in the feed (Wang et al., 1998), which may also explain why 50%

difference in BWG and 63% difference in FCR could happen when different kinds of phytobiotics are used in chicken diet (Xing, 2004). Although phytobiotics are a group of natural additives, research into their mechanisms of action, compatibility with diet, toxicity and safety assessment (based on the fact that some phytobiotic might have harmful substances) needs to be done before they can be applied more extensively in poultry feed. The results of the investigation by Fallah et al. (2013) showed that adding phytobiotic compounds as artichoke leaves meal and menthe extract in broiler diets caused a decrease in HDL and LDL concentration. Essential oils function mainly as antimicrobials and antioxidants; their antimicrobial ability may modulate the gut ecosystem to affect fat digestibility (Lee et al., 2004), starch or/and protein digestibility of feeds (Hernandez et al., 2004). A commercial preparation of essential oil components reduced faecal *Clostridium perfringens* counts of broilers in a field study (Mitsch et al., 2002).

ACIDIFIERS

Acidifiers have been used in poultry nutrition for long time, in different forms and combinations which are constantly changing. Organic acids reduce pH value of food and in this way act as conserving agents and prevent microbiological/microbial contamination of food, and this effect is exhibited also in digestive tract of poultry (Eidelsburger and Kirchgessner, 1994). Several mechanisms through which dietary acids may produce desired effects have been proposed (Partanen, 2001); reduced gastric pH, reduced survival of pathogens through the stomach, increased digestion of nutrients and direct killing of bacteria appear to be the most prominent organic acids in undissociated form are lipophilic and can diffuse across bacterial cell membranes to reach the interior of the cell. In a relatively high intracellular pH, they dissociate and disrupt the bacterial cell function. The effect may be stronger in some bacteria than in others (Partanen, 2001). In brief, acids dissimilarly affect the microbial populations along the digestive tract. In the stomach, the numbers of coliforms and *E. coli* increase regardless of type and form of organic acid, but there is no clear-cut evidence about the effect of acidifiers on *Lactobacillus* population. Acids generally reduce the populations of *Lactobacillus* in the intestines and *E. coli* in the colon. It appears that addition of acidifiers to the diet may not result in an environment that is favorable for beneficial bacteria like *Lactobacillus* but adverse to coliforms and *E. coli* (Chapman, 1988). Favourable effect of supplementation of individual organic acids to mixtures was established relatively long time ago for formic acid (Kirchgessner et al., 1992) and fumaric acid (Vogt et al., 1981). In research published by Ao et al. (2009) it was established that citric acid in combination with α -galactosidase increased the effect of enzyme action, but also had negative effect of feed consumption and gain.

Acidifiers, particularly the short chain fatty acids, acetate, propionate and butyrate have contributed greatly to the profitability in poultry and also provide people with health and nutritious poultry products (Patten and Waldroup, 1998). Moreover, acidifier improved growth performance through establishment of low gastrointestinal pH condition by supporting endogenous digestive enzymes and reducing undesired gut microorganism. Acidification of diet with weak organic acids such as formic, fumaric, propionic, lactic and sorbic acids have been reported to decrease colonization of pathogen and production of toxic metabolites, improved digestibility of protein, Ca, P, Mg, Zn and served as substrate in the intermediary metabolism (Richards et al., 2005).

CONCLUSION

Withdrawal of antibiotics from poultry foods created need for alternative solutions which would influence improvement of health and production traits of broiler chickens. Alternative growth promoters are probiotics, prebiotics, synbiotic, acidifiers and phytogetic additives. By increasing the growth of beneficial microbes or by reduction and removal of potential pathogens, the alternatives to IFAs possibly can improve the health and performance of birds. However, their effects on gut micro flora interact with digestive physiology and thus growth in many complex ways, which can be further influenced or even determined by many other factors such as the compatibility between the diet and the alternative, hygiene standards and animal husbandry practices. There possibly remain many questions to be answered or barriers to be overcome so that the alternatives can be applied (more) successfully in the industry in future.

REFERENCES

- Anonymous (2002). Guidelines for the Evaluation of Probiotics in Food. Report of a Joint FAO/WHO Working Group on Drafting Guidelines for the Evaluation of Probiotics in Food. London Ontario, Canada, 30 April and 1 May 2002.
- Ao T, Cantor AH, Pescatore AJ, Ford MJ, Pierce JL, Dawson KA (2009). Effect of enzyme supplementation and acidification of diets on nutrient digestibility and growth performance of broiler chicks. *Poult. Sci.* 88:111-117.
- Awad WA, Ghareeb K, Bohm J (2008). Intestinal structure and function of broiler chickens on diets *faecium* and oligosaccharides. *Int. J. Mol. Sci.* pp. 2205-2216.
- Bailey JS, Blankenship LC, Cox NA (1991). Effect of fructo-oligosaccharide on Salmonella colonization of the chicken intestine. *Poult. Sci.* 70:2433-2438.
- Barton MD (1999) The down-side of antibiotic use in pig production: The effect of antibiotic resistance of enteric bacteria. In manipulating Pig Production VII (ed PD Carnwell). Aust. Pig Sci. Association.
- Bellisle F, Diplock AT, Hornstra AT (1998). Functional food science in Europe. *Br. J. Nutr.* 80:3-4.
- Bhat GA, Khan AA, Mattoo FA (2003). Use of probiotic feed supplement in high fiber broiler ration. *Poultry Guide*, 18-20.
- Chang J (2000) Medicinal herbs: drugs or dietary supplements? *Biochem. Pharmacol.* 59:211-219.
- Chapman JD (1988). Probiotics, acidifiers and yeast culture a place for natural additives in pig and poultry production. Proceedings of Alltech's Fourth Annual Symposium. pp. 219-233.

- Collins MD, Gibson GR (1999). Probiotics, prebiotics, and symbiotics: approaches for modulating the microbial ecology of the gut. *Am. J. Clin. Nutr.* 69:1052S-1057S.
- Cowan MM (1999). Plant products as antimicrobial agents. *Clin. Microbiol. Rev.* 12:564-582.
- Cross DE, Mcdevitt RM, Hillman K, Acamovic T (2007). The effect of herbs and their associated essential oils on performance, dietary digestibility and gut microflora in chickens from 7 to 28 days of age. *Br. Poult. Sci.* 48:496-506.
- Cummings JH, Macfarlane GT (2002). Gastrointestinal effects of prebiotics. *Br. J. Nutr.* 87(2):145-151.
- Dibner JJ, Buttin P (2002). Use of organic acids as a model to study the impact of gut microflora on nutrition and metabolism. *J. Applied Poult. Res.* 11:453-463.
- Eidelsburger U, Kirchgessner M (1994). Zum Einfluß organischer Säuren und Salze im Futter auf die Mastleistung von Broilern. *Archiv für Geflügelkunde.* 58:268-277.
- Fallah R, Kiani A, Azarfar A (2013). Effect of Artichoke leaves meal and menthe extract (*Mentha piperita*) on immune cells and blood biochemical parameters of broilers. *Global Veterinarian.* 10(1):99-102.
- Fallah R, Rezaei H (2013). Effect of dietary prebiotic and acidifier Supplementation on the growth performance, carcass characteristics and serum biochemical parameters of broilers. *J. Cell Anim. Biol.* 7(2):21-24.
- Fuller R (1989). Probiotics in man and animals. *J. Appl. Bacteriol.* 66:365-378.
- Fuller R (1992). Probiotics. The Scientific Basis. Chapman and Hall, London.
- Fuller R (1997). The importance of Lactobacilli in maintaining kormal microbial balance in the crop. *Br. Poult. Sci.* 18:85-94.
- Gauthier R (2002). (XVIII Congreso Lantioamericano de Avicultura) (2002) Poultry Therapeutics: New alternatives. Accessed in 2006. http://www.jefo.ca/pdf/ALA2003_en.pdf.
- Georgieva V, Denev ST, Marinov B (2000). Effect of some probiotic and nutritive means on chicken broiler productivity. *Zhivotnov"dni Nauki.* 37:19-23.
- Gibson GR, Roberford MB (1995). Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. *J. Nutr.* 125:1401-1412.
- Green AA, Sainsbury DWB (2001). The effects of enzyme and probiotic supplementation to diets on broiler performance. *Turk. J. Vet. Anim. Sci.* 11:895-903.
- Hennig A, Ludke H, Schone F, Meixner B (1993). Some aspects of the use and evaluation of substances stabilizing intestinal microorganisms. *Poult. Abstr.* 19:1312.
- Hernandez F, Madrid J, Garcia V, Orengo J, Megias MD (2004). Influence of two plant extracts on broiler performance, digestibility and digestive organ size. *Poult. Sci.* 83:169-174.
- Jin LZ, Ho YW, Abdullah N, Jalaludin S (1998). Growth performance, intestinal microbial population and serum cholesterol of broiler fed diets containing Lactobacillus cultures. *J. Poult. Sci.* pp. 1259-1265.
- Kirchgessner M, Gedek B, Wiehler S, Bott A, Eidelsburger U, Roth FX (1992). Influence of formic acid, calcium formate and sodium hydrogen carbonate on the micro flora in different segments of the gastrointestinal tract. *J. Anim. Physiol. Anim. Nutr.* 68:73-81.
- Lee KW, Ecerts H, Beynen AC (2004). Essential oils in broiler nutrition. *Int. J. Poult. Sci.* 3:738-752.
- Lilley DM, Stillwell RH (1965). Probiotics: Growth promoting factors produced by microorganisms. *Poult. Sci.* 147:747-748.
- Liong MT, Shah NP (2006). Effects of a Lactobacillus Casei symbiotic on serum Lipoprotein, intestinal microflora and organic acid in rats. *J. Dairy. Sci.* 89:1390-1399.
- Maiorka A, Santin E, Sugeta SM, Almeida JG, Macari M (2001). Utilization of prebiotics, probiotics or symbiotics in broiler chicken diets. *Revista Brasileira de ciencia avicola.* 3:75-82.
- Mandal SK, Biswas TK, Mandal L (1994). Efficiency of different growth promoters on the performance of broilers. *Indian J. Poult. Sci.* 29:13-17.
- Mateo CD, Jacques KA, Harvez J (2000). Organic chromium, mannan oligosaccharides and zinc bacitracin: Effect on broiler performance and carcass characteristics. *Poult. Sci.* 79:116.
- Mitsch P, Kohler B, Gabler C, Losa R, Zitterl-Eglseer K (2002). CRINA poultry reduces colonisation and proliferation of Clostridium perfringens in the intestine and faeces of broiler chickens. Abstracts from the Eleventh European Poultry Conference, Bremen, Germany.
- Mohnl M, AcostaAragon Y, Acostaojedu A, Rodriguessanches B, Pasteiner S (2007). Effect of symbiotic feed additive in comparison to antibiotic growth promoter on performance and health status of broilers. *Poult. Sci.* 86(1):217.
- Nahashon SN, Nakave HS, Mirosh LW (1996). Performance of single comb white leghorn lagers fed with a live microbial during the growth and egg lagging phases. *Anim. Feed. Sci. Technol.* 57:25-38.
- Palod J, Singh VS (2004). Role of probiotics in Broiler feeding. Sadana's All India poultry Business Directory (Year Book 2003-2004) Special Millennium issue, 2nd ed. Sadana publishers and Distributors, Ghaziabad. pp. 147-148.
- Panda AK, Reddy MR, Rao SVR, Raju, MVL, Praharaj NK (2000). Growth, carcass characteristics, immune competence and response to *Escherichia coli* of broilers fed diets with various levels of probiotic. *Archiv fur Geflugelkunde.* 64:152-156.
- Parker R (1974). Probiotics, the other half of the antibiotic story. *Anim. Nutr. Health.* 28:240-255.
- Partanen K (2001). Organic acids their efficacy and modes of action in pigs. *Gut Enviroment of Pigs.* Nottingham University Press. pp. 201-218.
- Patten ID, Waldroup P (1998). Use of organic acid in broiler diets. *Poult. Sci.* 67:1178-1182.
- Patterson JA, Burkholder KM (2003). Application of prebiotics and probiotics in poultry production. *Poult. Sci.* 82:627-631.
- Rezaei, H, Jafari Khorshidi K, Fallah R (2013). The Effect of Feeding primalac probiotics on growth performance and blood parameters of ostriches. *Mal. J. Anim. Sci.* 16(1):79-86.
- Richards JD, Gong J, Delange CEM (2005). The gastrointestinal microbial and its role in monogastric nutrition and health with an emphasis on pigs; current understanding possible modulations and New technologies studies. *Can. J. Anim. Sci.* 85:421-435.
- Ten Bruggencate SJM, Bovee-Oudenhoven IMJ, Lettink-Wissink MLG, Van Der meer R (2003). Dietary fructo-oligosaccharides dose-dependently increase translocation of salmonella in rats. *J. Nutr.* 133:2313-2318.
- Vidanarachchi JK, Mikkelsen LL, Sims IM, Iji, PA, Choct M (2006). Selected plant extracts modulate the gut microflora in broilers. *Proceedings of Australian Poultry Science Symposium, Sydney, Australia.* 18:145-148.
- Vogt H, Matthes S, Harnisch S (1981). Der Einfluß organischer Säuren auf die Leistungen von Broilern und Legehennen. *Archiv für Geflügelkunde.* 45:221-232.
- Vranesic N (1992). Probiotics- useful bacteria as feed additives. *Poult. Abstr.* 18:1-5
- Wang R, Li D, Bourne S (1998). Can 2000 years of herbal medicine history help us solve problems in the year 2000? Biotechnology in the feed industry: Proceedings of Alltech's 14th Annual Symposium, Kentucky, USA. pp. 273-291.
- Windisch W, Kroismayr A (2006). The effects of phytobiotics on performance and gut function in monogastrics. Accessed in 2006. www.feedinfo.com.
- Xing XL (2004). A comparative study on the effects of different Chinese herbal medicinal feed additives in broiler chickens. Accessed in 2006.
- Xue M, Meng XS (1996). Review on research progress and prosperous of immune activities of bioactive polysaccharides. *J. Tradition. Vet. Med.* 3:15-18.
- Yusrizal C, Chen TC (2003). Effect of adding chicory fructans in feed on broiler growth performance, serum cholesterol, and intestinal length. *Int. J. Poult. Sci.* 3:214-219.
- Zhan XA, Hu CH, Xu ZR (2003). Effects of fructo-oligosaccharide on growth performance and intestinal microflora and morphology of broiler chicks. *Zhong Guo Shou Yi Xue Bao* 32:196-198.
- Zhang G, Ma L, Doyle MP (2006). Efficiency of probiotics, prebiotics and synbiotics on weight increase of chickens. (*Gallus Domesticus*). <http://www.ugacfs.org/research/pdfs/Poultry2006.pdf>.
- Zikic D, Peric L, Uscebrka G, Milosevic N, Jotanovic S (2006). Probiotici i prebiotici u ishrani brojlera: 1. Efekat na proizvodne rezultate. 11. Savetovanje biotehnologiji, Čačak, 11(2):471.

Full Length Research Paper

Participatory epidemiology and associated risk factors of foot-and-mouth disease in cattle in South Omo zone, South-Western Ethiopia

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Accepted 10 September, 2013

Participatory appraisal was applied to determine the major cattle diseases and investigate the epidemiology of foot-and-mouth disease (FMD) in South Omo pastoral and agro-pastoral livestock production system. Furthermore, assessment of associated risk factors in particular concern to local production system was conducted by participatory appraisal. The participatory methods used were clinical observation, matrix ranking and scoring, proportional piling and seasonal calendar. The result of matrix ranking and scoring showed that hemorrhagic septicemia, contagious bovine pleuro pneumonia (CBPP), trypanosomosis, FMD, black leg and anthrax were the major diseases of cattle in South Omo zone. The finding of seasonal calendar indicated that incidence of FMD was found to be high during the dry season than cold dry season. The lowest incidence was reported during the rainy season. Similarly, contact of herds with wild animals increased during dry season than rainy season. Based on this finding participatory epidemiology was found to be an important approach in veterinary investigations and it can also be used besides to conventional approaches.

Key words: Foot-and-mouth disease (FMD), participatory appraisal, South Omo zone, cattle, risk factors.

INTRODUCTION

In Africa, the non-forested portion of humid zone and the sub-humid part of sub-Saharan Africa are the areas with the greatest opportunity for expanding crop and livestock production. In this part of Africa, the greater half of the ruminant population are reared (over 232 million heads of cattle and 343 million heads of sheep and goats) (FAOSTAT, 2005).

This huge continental resource plays multi-factorial roles in mixed crop-livestock farming system. Hence, it has been proved that livestock production through improved production strategies could play a significant role in the development of a sustainable and environmentally friendly agriculture in efforts to ensure food security, poverty alleviation and effective utilization of natural resources (Afework et al., 2004; Shaw, 2004).

Economical loss due to occurrence foot-and-mouth disease (FMD) is tremendous that occurred due to death of young animals, marked reduction in milk yield, abortion in advanced stage of pregnancy and reduced working ability of the animals, reduced quality and quantity of meat, reduction in fertility, loss of quality of semen in breeding bull, etc., and the disease also restricts the possible export of livestock and livestock products (Yadav, 2003).

The poor farmers are most sufferers by FMD, because of non-availability of vaccine (FAO and OIE, 2012) and lack of awareness about vaccination program (Singh et al., 1987). Furthermore, the economic losses due to FMD were more to the marginal farmers. In Ethiopia, outbreaks of FMD frequently occur in the pastoral herds (Sahle,

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2004). This is mainly due to lack of effective vaccine, absence of livestock movement control and absence of systematic disease surveillance and reliable epidemiological data. However, it is likely that the disease is under-reported due to comparatively high tolerance of local breeds to the clinical episodes of the disease (Leforban, 2005). Therefore, it seems that FMD is more prevalent and has been one of the major causes for considerable economic loss of the rural communities in Ethiopia. Providing veterinary services to the communities according to the western model has proven difficult due to lack of infrastructure and the veterinarian has limited experience in harsh environments of pastoral system. Thus, participatory approach methods become important in disease investigations and in implementing different disease prevention and control strategies in developing countries, particularly in working with rural and pastoral communities. This is also of paramount importance to draw the indigenous knowledge and reach experience of the communities on the diseases of their locality (Leyland, 1991).

Furthermore, there is extensive traditional or ethno-veterinary knowledge that pastoralists have been known to possess and on which they rely to diagnose or treat many diseases (Rufael et al., 2006). It is possible that the proper collection and analysis of data for diseases like FMD that are often under reported by conventional veterinary services. Therefore, data collection in pastoral areas can better be managed in participatory manners. Participatory rural appraisal (PRA) is a systematic data gathering activity carried out by a multidisciplinary team to reveal the unidentified facts about a community (Lelo et al., 1995). Veterinarians and livestock workers have used and are presently using a variety of PRA methods to investigate animal health problems (Catley, 1997). The tools include interviewing, scoring and ranking, and visualization such as seasonal calendars, maps, Venn diagrams and flow charts.

Therefore, this study is designed with the objectives to determine the mortality and morbidity of major livestock diseases as compared to FMD and to assess the perception and understanding of community about the disease in the area.

MATERIALS AND METHODS

Study area

The study was conducted in two districts of South Omo zone, which are Dasanech and Hammer. The districts were selected from the existing districts of South Omo zone purposively; based on their geographical location, proximity to livestock market, huge ruminant population density, and possibility of contact with wild animals in nearby parks and sanctuary and to include different agro-ecologies of the zone. South Omo zone is located in South-West of SNNPR state, bordering with from east: Konso and Derashe Special districts of SNNPR state and Borena zone of Oromia regional state; North: North Omo and Kaffashaka zones; West: Sudan and Bench-maji zones; and South: Kenya. The zone covers an area of 22,000 km²,

and the area is inhabited by 14 ethnic groups, namely Ari, Banna, Hammer, Dasanech, Gngangatom, Kara, Kweegu, Mursi, Bodi, Malle, Tsemai, Arbore, Bacha and Dime (CSA, 2008). The study area is indicated in the map of Ethiopia (Figure 1).

Study animals and sampling technique

The study herds were selected from animal population in two districts of South Omo zone, Bennatsemay and Dasanech. From the selected districts, pastoral/peasant associations (PA) were selected randomly from the district's PA list. For this purpose, 10% of PA from the district was selected. From selected PA cattle herds were selected as primary sampling unit. Herd selection was implemented conveniently giving effort to include different herd sizes and composition in the PA. About 20% of cattle herds from the selected PA's were included in sampling. Individual animals from the selected herd were selected randomly and about 10% of animals in the herd were sampled to represent herd. The selected animals were observed for the clinical and chronic FMD.

Participatory appraisal of major livestock health problems were employed in each selected PA of the district. For every PA, which is considered as one community group, one appraisal was done.

Study design

Multistage sampling technique was employed to select study districts, PA, herd and sampling animal. Participatory appraisal method was applied to generate information on major cattle diseases and the epidemiology of FMD in the study area. The association regarding FMD in the area to contact history of wild life was thoroughly assessed. The participatory appraisal methods used were clinical observation, matrix ranking and scoring, proportional piling, pair-wise ranking and seasonal calendar.

Participatory disease appraisal

Clinical observation

Clinical observation of sick animals related to FMD was done during sample collection in order to cross check the perception of the communities with other participatory appraisal results. All sampling herds were visited for acute or chronic clinical signs. Clinical signs considered were vesicular to abrasion lesions in the oral cavity, lesions in inter-digital space, lameness and lesions in and around mammary glands and the teats, hair over growth and panting.

Matrix and pair wise ranking and scoring

Matrix scoring was conducted to understand the perception of community to major cattle diseases prevailing in the area. For this purpose, the selected community groups were made to select, rank and score the major five cattle diseases of the area. The ranking and scoring were done by matrix ranking and scoring and triangulated by pair-wise ranking and scoring. The ranking and scoring were done in eight community groups from eight selected PAs in Hammer and Dasanech districts of the study area. Group composition is made to include different community members by sex, age, skill, experience and social status for all participatory approaches done with groups.

Proportional piling

Proportional piling was employed in two districts, Hammer and Dasanech, representing different production system, to estimate the

SOUTH OMO ZONE

Southern Nations Nationalities Peoples Regional State

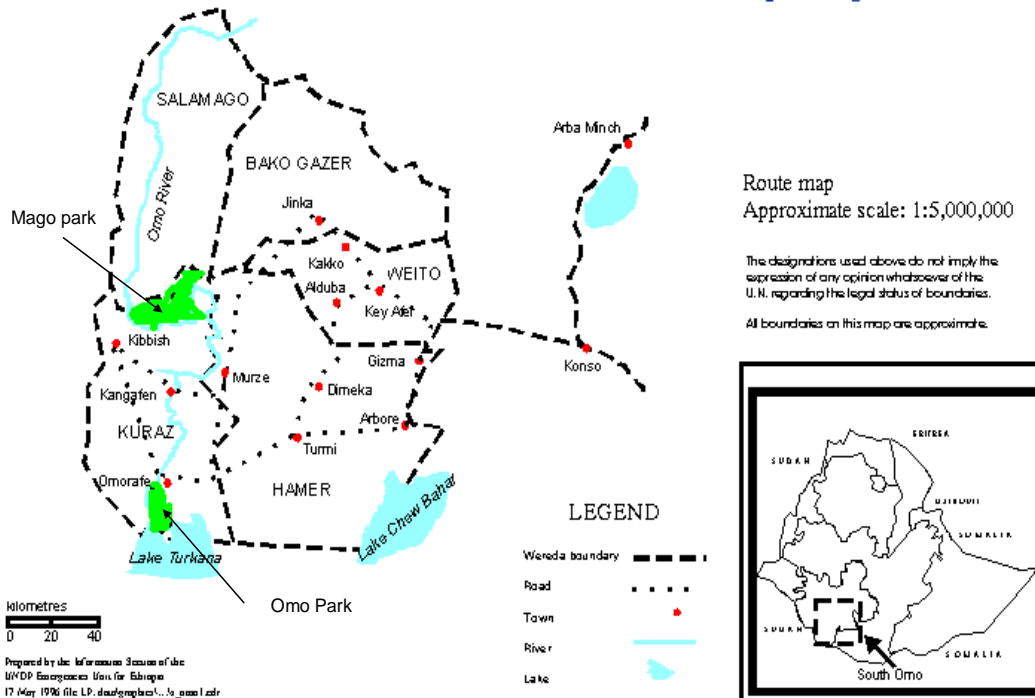


Figure 1. The study area, South Omo zone.

relative incidence and mortality caused by the five most important cattle diseases during the past one year, identified in matrix pairwise ranking and scoring earlier. Informant groups from selected PAs of each district were requested to classify the animals into different age groups and were classified into 3 categories. One age category of less than 1 year of age, between 1 and 4 years, and above 4 years. Then every informant group was allowed to maintain a pile of 100 stones for each age group. Then they were asked to separate proportion of pile representing sick animals from total herd for each age categories. This was done for major five diseases identified in matrix scoring and ranking for the last one year. Furthermore, the proportion of stones representing dead animals from sick animals was separated from the proportion of piles of stones representing sick animals. These were done separately for each age groups and different diseases to avoid confusion. Then, the piles of stones representing sick and dead animals from various diseases on different age groups were calculated to determine the incidence and mortality of various diseases in the area.

Seasonal calendar

Seasonal calendars were used to determine the seasonal occurrence of FMD. Seasonal calendar of FMD and contact of cattle to wild animals were done in eight community informant groups from eight PA of Dasanech and Hammer districts. The different seasons of the year were categorized according to the pastoralist's division category in four categories as long rainy season, ranging from March to May; cold dry season, ranging from June to August; short rainy season, ranging from September to November; and long dry season, ranging from December to February.

RESULTS

Clinical observation

Nine sick animals with acute clinical signs were observed during the study period in 2 outbreaks, 1 in Bori PA of Bennatsemay district and another in Lalla PA of Hammer district. Both outbreak cases were reported at the end of the drought season. In both Pas, cattle with clinical FMD cases were reported that they were kept in Mago National Park and were brought to the homestead. Chronically sick animals from FMD were reported to be attacked by hyenas and other predators in the park area, because these animals cannot escape due to lameness and weakness by the disease and feed shortage aggravated by drought.

Common clinical signs observed in acutely affected animals from FMD were vesicular to abrasive lesions in the oral cavity, frothy discharge from the mouth, lesion in the interdigital space and on the teats. Shade seeking and standing inside and not willing to come out of watering points is a typical feature of the disease, according to the pastoralist's knowledge. Young animals usually die due to FMD virus infection.

However, the common cause of death of FMD infected adult cattle was an attack by predators in South Omo zone. According to pastoralist's observation, those animals which survived acute FMD showed overgrowth of

hair, dullness, isolation from the herd, standing under shade during hotter hours of a day and reduced milk production.

Matrix/pair wise ranking and scoring

The summarized major five diseases of cattle identified and prioritized by four pastoralist informant groups, in four PAs in Dasanech district were contagious bovine pleuro pneumonia (CBPP), septicemic pasteurellosis, anthrax, FMD and black leg. CBPP and septicemic pasteurellosis were found to be more important than other diseases due to their high case fatality rates in Dasanech district. The result in Hammer district showed that septicemic pasteurellosis, trypanosomosis, black leg, CBPP and FMD are among the most important disease in the district. Septicemic pasteurellosis scored 2 times more important than trypanosomosis and black leg, but 6 times more important than CBPP and FMD. In this district, community groups ranked the major diseases according to the losses incurred by the diseases. This indicates that the knowledge of community to major diseases in the area is good and they well explained the disease problems prevailing in their locality.

Proportional piling

The summarized mean number of sick and dead cattle from the major diseases in Dasanech and Hammer districts are shown in Table 1. The summarized occurrence (age specific) of major diseases of cattle in Dasanech district was found to be 5.6 (3 to 8%), 3.3 (0 to 6%), 2.6 (0 to 5%), 2.3 (2 to 3%), and 1.3 (0 to 3%) for CBPP, septicemic pasteurellosis, anthrax, FMD and black leg, respectively.

The summarized mean number of cattle affected and died from the major disease in Hammer district in the last one year according to proportional piling is as shown in Table 1. The occurrence (age specific) of major diseases of cattle in this district was 5.3 (3 to 7%), 4.0 (3 to 5%), 3.0 (1 to 5%), 2.0 (2%), and 1.6 (1 to 3)% of septicemic pasteurellosis, trypanosomosis, black leg, CBPP and FMD, respectively.

Seasonal calendar

Summarized seasonal calendar for FMD and contact with wildlife in Dasanech and Hammer districts are as shown in Table 2. The incidence of FMD was found to be high during the dry season than cold dry season. The lowest incidence was reported during the rainy season. The informants also have stated that seasonal cattle movement occurs frequently during the dry season in search of good pasture and water, which also is directly associated to contact wild animals in these districts. This

pattern of seasonal movement (mainly in the dry season) also determines contact between different herds of domestic animals, and it is exacerbated by limited water sources.

The occurrence of FMD was found to be higher during the dry season followed by cold dry season in both districts. In Dasanech district, FMD occurs throughout the year but in Hammer district there were no FMD cases in the long rainy season. The seasonal occurrence of FMD in Dasanech and Hammer districts were indicated in Figures 2 and 3. Furthermore, the association of contact pattern of cattle to wild animals and the occurrence of FMD in cattle in Dasanech and Hammer districts using seasonal calendar as shown in Figures 4 and 5. The association in Hammer district was by far linearly associated than that of Dasanech district which could be due to the fact that the contact to wild animals in Hammer is more regular in that the animals migrate in search of pasture and water thereby making regular contact to wild animals as compared to Dasanech where they migrate to long distances but not directly linked to parks and sanctuaries and thus the contact to wild animals is not regular.

DISCUSSION

This study indicated that FMD was the fourth most important cattle health problem and an emerging disease in South Omo zone. The descriptions by most of the cattle owners showed that they well know most of the clinical presentations of the disease. Indeed most of the signs listed for FMD were consistent with what is indicated in different reports (Catley et al., 2001, 2004; et al., 1994). In Ethiopia, similar signs have been reported in pastoral cattle of Afar (Tadesse, 2003), Somali (Eshetu, 2003) and Oromia (Rufael et al., 2008) regional states.

The higher district level occurrence of the disease was observed in Hammer district than that of Dasanech. This could be due to the fact that higher contact pattern of domestic animals to wild animals and also their high migratory pattern in Hammer as compared to Dasanech district which are not as migratory and not as such huge wild ruminant population bordering the district. The higher prevalence of disease in Hammer could be accounted due to the fact that this district is a center for cattle market facilitating contact among cattle from different sources and this area is border transit for transporting animals for draft power from the potential livestock producer areas like Dasanech and Gnagatom areas, to mixed crop livestock producer areas like Debub Aari and Malle districts.

There is strong association of contact pattern to wild animals to domestic animals with the occurrences of FMD in the cattle. This finding is in agreement with the reports of Macaulay (1963) and Hedger (1981) who observed that many species of wild animals have been reported as having been infected with FMD virus and

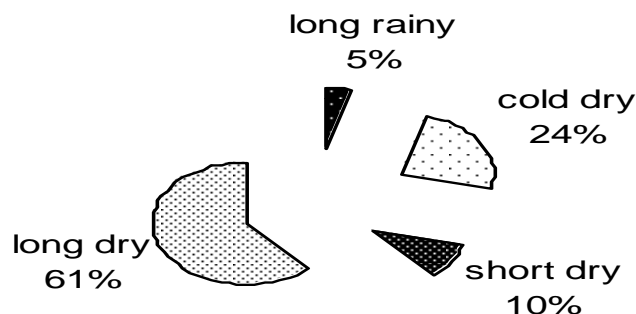
Table 1. Summarized age specific occurrence and mortality rates of major cattle diseases in Hammer and Dasanech districts by proportional piling.

District	Health status by age	Septicemic pasteurellosis	Trypanosomosis	Black leg	CBPP	FMD	Total
Hammer	Sick age less than 1	3	3	1	2	3	12
	Sick age 1 to 4	7	5	5	2	1	20
	Sick age above 4	6	4	3	2	1	16
	Died age less than 1	0	2	0	1	1	4
	Died age 1 to 4	2	1	2	0	0	5
	Died age above 4	3	0	0	0	0	3
	Healthy age less than 1	97	97	99	98	97	488
	Healthy age 1 to 4	93	95	95	98	99	480
	Healthy age above 4	94	96	97	98	99	484
	Subtotal	305	303	302	301	301	1512
Dasanech	Sick age less than 1	3	0	0	2	0	5
	Sick age 1 to 4	6	6	5	2	3	22
	Sick age above 4	8	4	3	3	1	19
	Dead age less than 1	0	0	0	1	0	1
	Dead age 1 to 4	2	3	2	0	1	9
	Dead age above 4	3	1	0	0	0	4
	Healthy age less than 1	97	100	100	98	100	495
	Healthy age 1 to 4	94	94	95	98	97	478
	Healthy age above 4	92	96	97	97	99	483
	Subtotal	305	304	302	301	301	1516
Total		610	607	604	602	602	3028

Table 2. Summarized seasonal calendar of FMD and contact of cattle to wild animals in Dasanech and Hammer districts.

District	Local seasonal category	Long rainy	Cold dry	Short rainy	Long dry
Dasanech	FMD occurrence	1	5	2	13
	Contact to wild animals	0	2	4	8
Hammer	FMD occurrence	0	4	4	12
	Contact to wild animals	2	3	6	15

FMD occurrence in Dasanech by season

**Figure 2.** Seasonal occurrence of FMD in Dasanech district.

FMD occurrence in Hammer by season

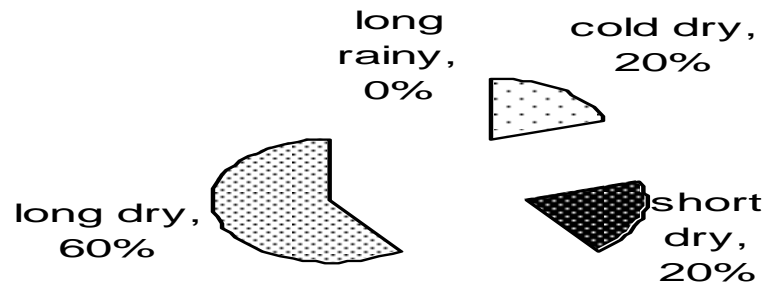


Figure 3. Seasonal occurrence of FMD in Hammer district.

Association of FMD and contact to wild animals, Dasanech

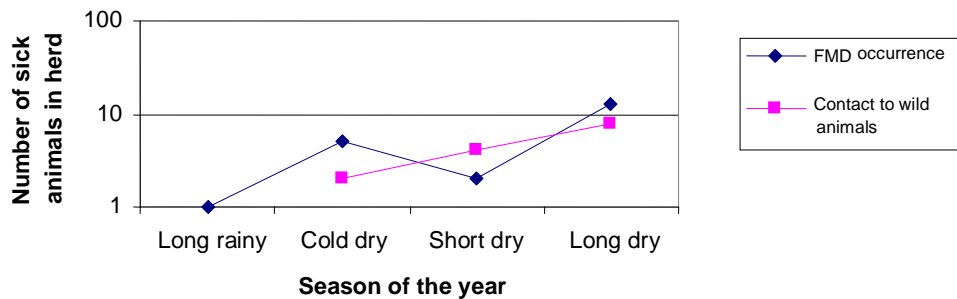


Figure 4. Contact pattern associated to FMD occurrence in Dasanech district.

Association of FMD and wild life contact, Hammer

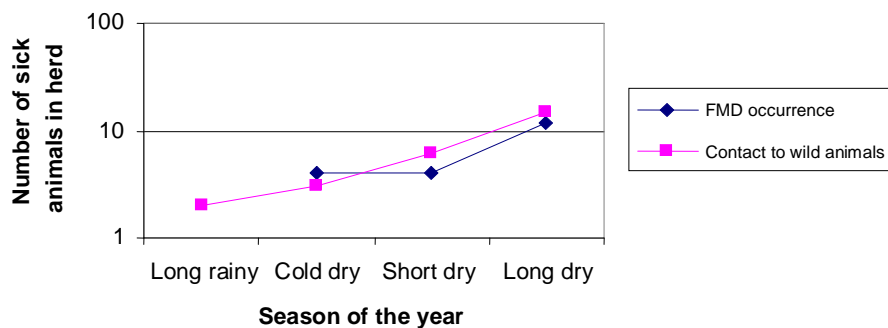


Figure 5. Contact pattern associated to FMD occurrence in Hammer district.

Furthermore, the serotypes circulating in South Omo zone could be different from those identified in other areas of the country and/or vaccinal strains available in Ethiopia, thus determining the serotypes in South Omo zone is of a primary step to be done for the control of FMD.

REFERENCES

Afework Y, Clausen PH, Abebe G, Tilahun G, Mehlitz D (2004). Multiple-drug resistant *Trypanosoma congolense* populations in village cattle of Metekel District, northwest Ethiopia. *Acta Trop.* 76:231-238.

Catley A (1997). Adapting participatory appraisal for the veterinary epidemiologists: PA tools for use in livestock disease data collection. SVEPM Proceedings, Chester, 1997.

- Catley A, Chibunda RT, Ranga E, Makungu S, Magayane FT, Magoma G, Madege MJ, Vosloo W (2004). Participatory diagnosis of heat intolerance syndrome in Cattle in Tanzania and Association with foot and mouth disease. *Prev. Vet. Med.* 65:17-30.
- Catley A, Okoth S, Osman J, Fison T, Njiru Z, Mwangi J, Jones BA, Leyland TJ (2001). Participatory diagnosis of a chronic wasting disease in cattle in southern Sudan. *Prev. Vet. Med.* 51:161-181.
- Condy JB, Herniman KAJ, Hedger RS (1969). Foot-and-mouth disease in wildlife in Rhodesia and other African territories. A serological survey. *J. Comp. Pathol.* 79:27-31.
- CSA (2008). Federal Democratic Republic of Ethiopia. Central Statistical Agency, Agricultural Sample Survey Report on Livestock and Livestock Characteristics. Volume II, 2007/08. Addis Ababa, Ethiopia.
- Eshetu T (2003). Participatory studies on heat intolerance syndrome associated with FMD in indigenous cattle of Somali pastoral area in Shinille Zone, Ethiopia. DVM, thesis, Addis Ababa University, Faculty of Veterinary Medicine, Debre Zeit.
- FAO, OIE (2012). The global foot and mouth Disease control strategy. Strengthening animal health systems through improved control of major diseases. FAO and OIE collaborative FMD control strategy.
- FAOSTATA (2005). Program for the control of African Animal Trypanosomosis and related development. Annual report of the year 2005. Food and Agricultural Organization of the United Nations, Rome.
- Hedger RS (1981). Foot-and-mouth disease. In: Infectious disease of wild animals, 2nd Ed. edited by Davis JW, Karstad LH, Traineder DO, 2nd edition. Ames, Iowa State University Press, Pp. 87-96.
- Leforban Y (2005). Report of a mission on foot and mouth disease in Ethiopia, Proposals for a strategic plan for a control program oriented to the export, 10-22 April, 2005. 12-42.
- Lelo F, Ayieco J, Makenzi P, Muhia N, Njeremani D, Muiruri H, Omollo J, Ochola W (1995). PRA field handbook for participatory rural appraisal reactionares. PRA program, Egerton University, Njoro, Kenya. pp.10-24.
- Leyland T (1991). Participation in the 80's and 90's. Who asks the questions in livestock development? Center for Tropical Veterinary Medicine. University of Edinburgh.
- Macaulay J (1963). Foot-and-mouth disease in non-domestic animals. *Bull Epizoot Dis Afr.* 11:143-146.
- May L.T, Condy J (1965). Foot-and-mouth disease in game in Rhodesia. *Bull. Off. Int. Epizoot.* 64:805-811.
- Radostits OM, Blood DC, Gay CC (1994). *Veterinary Medicine*, 8th Ed. London: BailliereTindall. pp 345-372.
- Rufael T, Catley A, Bogale A, Sahle M, Shiferaw Y (2008). Foot and mouth disease in the Borana pastoral system, southern Ethiopia and implications for livelihoods and international trade. *Trop. Anim. Health. Prod.* 40:29-38.
- Rufael T, Sahle M, Asseged B (2006). Participatory appraisal and seroprevalence study of Foot and Mouth Disease in Borana pastoral system, South Ethiopia. Masters of Science thesis. FVM, AAU, Debre Zeit, Ethiopia.
- Sahle M (2004). An epidemiological study on the genetic relationship on FMDV in east Africa. A thesis submitted in partial fulfillment of the requirement for the degree of Doctor of Philosophy in the department of veterinary tropical diseases, Faculty of Veterinary Science University of Pretoria, South Africa.
- Shaw APM (2004). Economics of African animal trypanosomosis. The trypanosomosis, pp. 369-402. CAB International, Wallingford, UK.
- Singh P, Sissodia B and Kunzru O (1987). An economic analysis of livestock diseases losses. *Indian Vet. J.* 64, pp. 227-230.
- Tadesse G (2003). Participatory studies on Heat intolerance syndromes Associated with FMD in indigenous cattle in Afar pastoral area of Ethiopia. DVM thesis. FVM, AAU, Debre Zeit, Ethiopia.
- Voslo W, Bastos ADS, Sangare O, Hargreaves SK, Thomson GR (2002). Review of the status and control of foot-and-mouth disease in sub-Saharan Africa. *Revue Scientifique et Technique de l'Office International des Epizooties* 21, 437-449.
- Yadav M (2003). Health barrier to buffalo productivity and their management. In: Proceedings of the 4th Asian Buffalo Congress on "Buffalo for Food Security and Rural employment, held at New Delhi, during February 25-28, 2003. 1:142-147.

Full Length Research Paper

Study on prevalence of hydatidosis and cyst characterization in camels (*Camelus dromedarius*) slaughtered at Akaki abattoir, Ethiopia

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Accepted 19 September, 2013

A cross-sectional study was conducted from October 2010 to August 2012 to determine the prevalence of camel hydatidosis and associated risk factors in camels slaughtered for human consumption at Akaki municipal abattoir. The results of this study revealed that out of 770 camels slaughtered, 474 (61.6%) were harboring hydatid cysts with varying numbers and sizes in different organs (3850) in the following manner; liver (53.51%), lungs (40.39%), heart (0.13%), gastrointestinal track (GIT, 0.9%) and Kidneys (0.13%). The positive samples were put in plastic bags and taken to the laboratory for characterization of the cysts for fertility and viability. There was no significant variation between camels of different origins and the anatomo-morphological features of the cysts. The infection rates varied significantly among age groups ($p < 0.05$), sex ($p < 0.005$) and body condition score ($p < 0.05$) of camels. The prevalence was found to be high (61.94%) in higher age group animals, that is, greater than 10 years as compared to 3 to 5 years (25%). The mean intensity of hydatid cyst among affected camels was found to be 3.1 ± 5.8 . Out of the examined cysts, 41.5% were found to be fertile and viable, while 18.3, 21.3 and 18.9% were non-viable, sterile and calcified cysts, respectively. The fertility of the cysts was 68.5 and 70.7% in liver and lungs, respectively. The high prevalence of camel hydatidosis affecting different organs indicates the seriousness of this disease particularly in the area of the origin of these animals that require an immediate control intervention.

Key words: *Camelus dromedarius*, cyst fertility, hydatid cyst, prevalence.

INTRODUCTION

Ethiopia is an agrarian country with huge livestock population in Africa possessing 23 millions heads of camels (MOI, 2005). Even though these animals play a crucial role in providing draught power, determining the wealth, social and food status of pastoralist living in mid-altitude and low land of Ethiopia, Africa and Asia, little is known about their husbandry practices, productive and reproductive performances (Getahun and Belay, 2002).

Among parasitic diseases affecting camels, hydatidosis

is a diseases with substantial economic and public health importance occurring in many countries (Lahmar et al., 2004) and is becoming more endemic in many African countries (Azlaf and Dakkak, 2006; Getaw et al., 2010).

Hydatidosis/Echinococcosis is a cosmopolitan zoonosis caused by larval stages of cestodes belonging to the genus *Echinococcus* (Craig et al., 2007). Larval infection (hydatidosis) is characterized by long term growth of hydatid cysts in the intermediate host. Factor governing

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the prevalence of hydatidosis in a given locality may be associated with prevailing specific social cultural, environmental conditions and the dynamics of transmission between the dog and its intermediate host and human (Macpherson, 1985). The public health and economic significances of hydatidosis lies on the cost of hospitalization, medical and surgical fees, loss of income and productivity due to permanent or temporary incapacity to work. The social consequence of hydatidosis is disability and mortality (Macpherson et al., 1985). In food animals, hydatidosis has an adverse effect on production causing decreased production of meat, milk, wool, reduction in growth rate and predisposition to other diseases (Kebede et al., 2009b).

Previous studies (Mohammed, 1988; Abdul-Jawed, 1988) from different parts of Ethiopia reported the prevalence of cattle and sheep hydatidosis ranging from 25.7 to 63% and 4.4 to 18.8%, respectively. However, few reports (Woldemeskel et al., 2001; Salih et al., 2011) are available on the prevalence of camel hydatidosis in Ethiopia. Therefore, this study was designed to estimate the prevalence of hydatidosis, determining cyst fertility, viability and assessment of the associated risk factors in slaughtered camels at Akaki abattoir.

MATERIALS AND METHODS

Study area

The study was conducted at Akaki abattoir, which is located in Addis Ababa, the capital city of Ethiopia. The city is located at 9°1'48' North and 38° 44'-24' East at an average altitude of 2500 m above sea level. The annual rainfall is about 800 to 1100 mm³ and a mean annual maximum and minimum temperature is about 21 to 27°C, respectively (NMSAE, 2012). Although the camel meat is not popular in Addis Ababa, the Somali community and some other Muslim communities who live in the city are the main consumers of camel meat from this abattoir. As a result the Akaki abattoir usually slaughters an average of eight camels per day. In addition, this abattoir, also give service to the hotels and restaurants of the Akaki town by slaughtering cattle, sheep and goats every day.

Study animals

The study was conducted on 770 one humped camels (*Camelus dromedarius*) brought from various camel rearing pastoral areas of the east and south part of the country, namely, Borena, Chiro, Metahara, Miesso and Kereyou.

Sample size determination and sampling procedure

It is reasonable to assume the systematic sample is a representative as a simple random sample. Therefore, the sample size was calculated using the formula given for simple random sampling (Thrusfield, 2005) with 50% expected prevalence, 95% confidence interval and 5% desired absolute precision. Accordingly, the calculated sample size was found to be 384. In order to maximize randomness as well as level of precision, a total of 770 camels which were slaughtered at Akaki abattoir for human consumption

were sampled. The sampling was carried out using systematic random sampling (Thrusfield, 2005) in such a way that the sampling units were selected at equal intervals with the first animal being selected randomly.

Study type and methodology

During the cross-sectional study, the ante mortem and post mortem inspection was carried out in accordance with the procedures of Ethiopian Ministry of Agriculture Meat Inspection Regulation 1972. During ante mortem inspection, the animals were placed in a collection barn for 24 h for visual observation. Animals which show clinical signs of illness and some pathological alterations, a checkup and treatment were carried out. Information concerning age, body condition score, sex, behavior, and nutritional status of all animals were properly recorded (Gracey et al., 1999). The age of the sampled animals was determined by dental eruption according to Khan et al. (2003). The body condition scoring for camels was carried out based on the guidelines given by Faye et al. (2001). The scoring was conducted by looking at the back and flank and then classified as poor (0 and 1), medium (2 and 3), and good (4 and 5).

Post mortem inspection

Regular visits were made to Akaki abattoir and a thorough examination of visceral organs (liver, lungs, heart, spleen, kidneys, other viscera and tissues) was done by inspection, palpation and incision of each slaughtered camels. The number of cysts and the organ from which the cysts recovered were also recorded systematically and collected in a plastic bag for close examination in the laboratory to confirm the doubtful cases immediately.

Determination of cyst fertility and viability

After collection of samples from each hydatid cysts positive organs, the wall of the cyst was punctured by a large needle and opened with scalpel blade and contents were transferred into test tube. Based on the presence and absence of broad capsule containing protoscolices in hydatid fluid, cysts were identified and classified as fertile and infertile. The infertile cysts were further classified as sterile (fluid filled cysts without protoscolices) and calcified, as indicated by Macpherson (1985). To determine viability of protoscolices, a drop of the sediment of the cyst fluid with protoscolices was placed on microscopic glass slide and covered by 22 × 22 cover slip and then an amoeboid peristaltic movement (flame cell activity) was observed under the objective of 40x (Smyth and Barrett, 1980). When it becomes confusing to observe such movement, a drop of 0.1% aqueous eosin solution was added to equal volume of protoscolices to completely or partially exclude the dye, the dead one took the dye (stained red), whereas the viable one were not stained (Macpherson, 1985).

Data analysis

The data obtained from post mortem and laboratory findings were entered to Ms Excel sheet and then analyzed by using statistical Package for Social Sciences (SPSS) for window version 15, SPSS, Inc, Chicago, IL. Initially, univariate logistic regression followed by multiple logistic regression analysis was employed to analyze the existence of association between the different risk factors and occurrence of hydatidosis. The 95% confidence intervals (CI) were also calculated. All value of P<0.05 were considered significant.

Table 1. Prevalence of camel hydatidosis based on age and Body condition score in Akaki abattoir.

Risk factors		No. of camels examined	No. infected camels	Prevalence (%)
Sex	Female	760	470	61.8
	Male	10	4	40
Age	3-5 years	8	2	25
	>10 years	762	472	61.9
BCS	Medium	712	434	61
	Good	58	40	69

Table 2. Prevalence of hydatid cysts based on anatomical sites.

Organs (Sites)	Total No. of organs examined	No. of infected organs	Prevalence (%)
GIT	770	7	0.90
Heart	770	1	0.13
Kidneys	770	1	0.13
Liver	770	412	53.51
Lungs	770	311	40.39
Total	3850	732	16.73

Table 3. Organ distribution of the hydatid cyst in each organ.

Organ	No. of organs infected	No. of cysts in each organ
GIT	7	15
Liver	412	3774
Lung	311	3132
Heart	1	1
Kidney	1	1
Total	732	6923

Table 4. Organ distribution of hydatid cysts on the basis of their size.

Organ	Small	Medium	Large	Total
GIT	4	2	1	7
Heart	0	1	0	1
Kidney	1	0	0	1
Liver	2150	1320	304	3774
Lung	1632	1217	284	3132
Total	3786	2540	589	6915

RESULTS

Prevalence and analysis of the risk factors

During the study period, from October 2010 to April 2012, a total 770 camels were examined, out of which 474 (61.6%) were found to be harboring hydatid cysts in their internal organs. The occurrence of hydatidosis significantly varied among age group ($P < 0.05$), sex ($P < 0.05$), and body condition score of camels ($P < 0.05$) (Table 1).

Out of 474 camels infected, the prevalence from infected cases count the highest relative percentage in liver 53.5% (412/770), lungs 40.39% (311/770), gastrointestinal track (GIT) 0.9% (7/770), heart 0.13% (1/770) and kidneys 0.13% (1/770) (Table 2).

Out of a total of 3850 organs examined for hydatid cysts, 732 organs were harboring cysts. The maximum

number of cysts found in infected organ was 32, while the minimum was 1. The mean intensity of cysts in camels harboring hydatid cysts was 9.16 (Table 3).

Organ distribution of the cyst

Single and multiple hydatid cysts distribution were recorded in different organs. Higher number of large and medium sized cysts was found in the lungs (Table 4), while higher number of small and calcified cysts was encountered in the liver (Tables 4 and 5). Most of the hydatid cysts were concentrated in liver and lungs of camels.

Cyst fertility and Viability

Out of 732 cysts observed and examined for fertility and

Table 5. Distribution of fertile (viable, nonviable), sterile and calcified hydatid cysts in liver and lungs of camels slaughtered at Akaki abattoir.

Organs inspected	No. of organs infected	Status of cysts			
		Fertile cysts		Infertile cysts	
		Viable	Non-viable	Sterile cyst	Calcified
Liver	412	165 (68.5%)	76 (31.3%)	84 (20.4%)	87 (21.1%)
Lung	311	135 (70.7%)	56 (29.3%)	70 (22.5%)	50 (16.1%)
Total	723	300 (41.5%)	132 (18.3%)	154 (21.3%)	137 (18.9%)

viability, 68.5% (165/723) were found to be viable and fertile in the liver, whereas 31.3% (76/723) were dead. However, in the lungs, 70.7% (135/723) viable and fertile and 29.3% (56/723) were dead and calcified once. Details of the percentage of viability and fertility of cysts in livers and lungs were indicated in Table 5.

DISCUSSION

In the present study, the prevalence of the hydatid disease was 61.56% which is higher than reports of Bitsat (2001) and Woldemeskel (2001). On the contrary, a very lower prevalence of camel hydatidosis was reported from Harar (Eastern Ethiopia) by Wubet (1987). These variations in results could be due to the variations in the temperature, environmental conditions, livestock health practice, the nature of the pastoral grazing and the way of upbringing these animals in the study areas.

In the present study, majority of the slaughtered and inspected animals were females than few numbers of male camels. Hence, more than 50% of inspected females were found to be positive for hydatidosis. Comparable findings have been reported in Kuwait (Abdul-salam and Farah, 1988). This might be related to the practices in the management of male and female camels that males are moved too far for grazing and watering, whereas females are usually managed around homesteads, at the backyard for milk purpose which commonly expose female animals to come in contact with infected dogs (Parija, 2004). In many camel breeding areas, offals are not consumed by the community rather given to dogs and this may increase the chance of environmental contamination, whereby dogs can easily acquire the infection and then continuously discharge eggs of *Echinococcus* parasites. Consequently, as females remain longer than males for reproductive purposes in the area, the probability of getting more infection will be higher than male ones. Moreover, the situation becomes more exacerbated as dogs are not kept in-door for religious and traditional matters resulting in increased number of stray dogs favours further dissemination of the disease.

The result showed that the infection prevalence was higher in the older age (10 years) classes ($P < 0.05$). The

age-dependent increase in infection rate among examined animals is in accordance with the findings of Azlaf and Dakkak (2006). The age variation can also be associated with differences in exposure to infection, because older livestock may have been exposed to more infective stages (Ibrahim et al., 2011). It is also possible to relate this general fact that most of the camels are slaughtered in their old age when their milk and/or calf production and working capacity get reduced and they fall sick frequently.

Among hydatidosis positive camels, liver (53.51%) was found to be the most frequently infected visceral organs, followed by lungs (40.31%), other organs (0.91%), heart (0.13%) and kidneys (0.13). This result is in agreement with other studies associated with camels (Woldemeskel et al., 2001) in Ethiopia, Ahmadi (2005) from Iran and Ibrahim and Craig (1998) in Libya. On the contrary, Ibrahim and Craig (1998) documented a high liver infection than lungs in their works. The observed higher frequency of infection in liver might be attributed to the great capillaries network present in liver that could easily trap circulated oncospheres (Getaw et al., 2010).

The fertility rate among the organs was found higher in lungs (70.68%) compared to liver which was 68.46%. It has been stated that the relatively softer consistency of lung tissue allows the easier development of the cyst (Himonas, 1987). Our result was also in agreement with the reports of Ahmadi (2005) from Iran who demonstrated that the fertility of the cyst from lungs was 69.7% as compared to 58.7% from liver in slaughtered camel in five different abattoirs. The greater prevalence and high fertility rate of pulmonary cyst over hepatic cyst of camel indicate the importance of internal organs as a potential source of infection to dogs.

Conclusively, hydatidosis was found to be one of the most important parasitic diseases in camels slaughtered at Akaki abattoir. The distributions of hydatid cyst in different organs implicate the seriousness of this disease in camel health. Further epidemiological studies on the diseases status, public health importance and associated risk factors influencing the occurrence of camel hydatidosis should be conducted in the areas of origin of these animals. The high prevalence reported in the current study warrant serious attention for prevention and control of this disease.

REFERENCES

- Abdul-Jawed A (1988). Hydatidosis prevalence at Jimma abattoir. DVM thesis, Faculty of Veterinary Medicine, Addis Ababa University. pp: 56-59.
- Abdul-salam JM, Farah MA (1988). Hydatidosis in camels in Kuwait. *Parasitol. Res.* 74:267-270.
- Ahmadi NA (2005). Hydatidosis in camel (*Camelus dromedarius*) and their potential role in the epidemiology of *Echinococcus granulosus*. *Iran. J. Helminthol.* 79:119-125.
- Azlaf R, Dakkak A (2006). Epidemiological study of cystic echinococcosis in Morocco. *Vet. Parasitol.* 137:83-93.
- Bitsat K (2001). Prevalence of hydatidosis in Jijiga municipal abattoir. DVM thesis. Jimma University, Ethiopia.
- Craig PS, McManus DP, Lightowers MW, Chabalgoity JA, Garcia HH, Gavidia CM, Gilman RH, Gonzalez AE, Lorca M, Naqira C, Nieto A, Schantz PM (2007). Prevention and control of cystic echinococcosis. *Lancet. Infect. Dis.* 7:385-394.
- Faye B, Bengoumi M, Cleradin A, Tabarani A, Chilliard Y (2001). Body condition score in dromedary camel: A tool for management of reproduction. *Emir. J. Agric. Sci.* 13:1-6.
- Getahun T, Belay K (2002). Camel husbandry practices in Eastern Ethiopia; The case of Jijiga and Shinile zone. *Nomadic People* 6:155-176.
- Getaw A, Beyene D, Ayana D, Megersa B, Abuna F (2010). Hydatidosis prevalence and its economic importance in ruminants slaughtered at Adama municipal abattoir. Central Oromia, Ethiopia. *Acta Tropica.* 113:221-225.
- Gracey JF, Collins DS, Huey RJ (1999). *Meat hygiene*, 10th Edition, Baillière Tindall, London.
- Himonas C (1987). The fertility of hydatid cyst in food animals in Greece, *Helminth Zoonosis*, Martinus Nijhoff Publishers, Netherland.
- Ibrahim MM, Craig PS (1998). Prevalence of cystic Echinococcus in camels (*camelus dromedaries*) in Libya. *J. Helminthol.* 72:27-31.
- Ibrahim K, Thomas R, Peter K, Omer RA (2011). A molecular survey on cystic Echinococcosis in Sinnar area, Blue Nile state (Sudan). *Chin. Med. J.* 124(18):2829-2833.
- Kebede W, Hagos A, Girma Z, Lobago F (2009b). Echinococcosis / Hydatidosis; its prevalence, economic and public health significance in Tigray region, Northern Ethiopia. *Trop. Animal. Health. Prod.* 41:865-871.
- Khan BB, Iqbal A, Riaz M (2003). Production and management of camels. University of Agriculture, Faisalabad, Pakistan. pp. 152-156.
- Lahmar S, Debbek H, Zhang LH, McManus DP, Souissi A, Chelly S, Torgerson PR (2004). Transmission dynamics of the *Echinococcus granulosus* sheep-dog strain (GI genotype) in camels in Tunisia. *Vet. Parasitol.* 121(1-2):151-156.
- Macpherson CNL (1985). Epidemiology of hydatid disease in Kenya. A study of the domestic intermediate hosts in Masuil and Traan. *Royal Soc. Trop. Med. Hyg.* 79(2):209-320.
- Ministry of Information (MOI) (2005). Expert products of Ethiopian press release of ministry of information, Department of press and audiovisual. Addis Ababa, Ethiopia.
- Mohammed A (1988). Study on the prevalence and economic significance of bovine Hydatidosis in Gamo-Goffa region. DVM thesis, Addis Ababa University, Faculty of Veterinary Medicine.
- National Meteorology Service Agency of Ethiopia (2012). Annual metrological analysis and report.
- Parija SC (2004). *Medical Parasitology, Protozoology and Helminthology. Text and Atlas 2nd Ed.* India Ichennia Medical Books Publisher. pp. 221 – 229.
- Salih M, Degefu D, Yohannes M (2011). Infection Rates, Cyst Fertility and Larval Viability of Hydatid Disease in Camels (*Camelus dromedarius*) from Borana, Kereyu and Harar Areas of Ethiopia. *Global Veterinaria.* 7(6):518-522.
- Smyth JD, Barrett NJ (1980). Procedure for testing the viability of human hydatid cysts following surgical removal, especially after chemotherapy. *Trans. Royal Soc. Trop. Med. Hyg.* 74:649-652.
- Thrusfield M (2005). *Veterinary Epidemiology*, 3rd Edition UK. Blackwell science Ltd. pp. 182-198.
- Woldemeskel M, Issa A, Mersic A, Potgieter LND (2001). Investigation of parasitic disease of one- humped camel (*Camelus dromedarius*) in eastern Ethiopia. *J. Camel Pract. Res.* 8:77-81.
- Wubet M (1987). A preliminary study of echinococcosis/hydatidosis in Haraghe region and the efficacy of *Glinhs lotoidus* seeds against *Echinococcus granulosus* in pups infected experimentally with hydatid material. DVM thesis, Addis Ababa University, Ethiopia.

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