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STUDY OF THE EFFECT OF OCCULTATION DIETARY *AZOLLA PINNATA* IN REDUCING FAECAL BACTERIAL LOAD IN RABBITS

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Abstracts

The experimental study was carried out in the animal farm of Veterinary Medicine, Baghdad University. Sixteen local rabbits males, at aged between 4.5–5 months and range weight 1.526 – 1.609 kg were used.T he animals were divided into four equal groups as following, G1served as control group was fed on basal diet, G2 group was fed on basal diet contain 5% *Azolla*, G3 group was fed on basal diet contain 10% *Azolla*, G4 group was fed on basal diet contain 15% *Azolla*. The objective of the work was to study the sustainable holds of *Azolla* in the rabbits feed to reduce the fecal total bacterial count and fecal coliform count. The results revealed non-significant in fecal bacterial count and fecal coliform count in all the treatment groups. The results indicated that the rabbits of control group had higher fecal bacterial count and fecal coliform count compared with G2, G3 and G4 groups. However, further study is required to make a reliable recommendation for trade rabbits' productivity.

Key words : *Azolla*, coliform count, fecal bacteria, rabbits.

Introduction

Azolla is a small aquatic fern, belonging to the family *Azollaceae* is an aquatic free floating fern (flows on the water surface). Referrers the name to conjugation of two Greek words, azo (to dry) and allyo (to kill) because the fern is killed by drought (Mishra *et al.,* 2016). Many species of *Azolla* can be found all over the world especially in tropical and subtropical regions. It easily grows in stagnant water, pond, canals and marshy lands as the pH water from 4-7. Pillai *et al.,* (2002) reported that the *Azolla piñata* is one of the good sources of protein and contains most of the essential amino acids minerals, such as iron, calcium, magnesium, potassium, phosphorus, manganese etc, apart from quantities of vitamin 'A' precursor beta-carotene and vitamin B12. It is also contains probiotics and biopolymers. Thus, *Azolla* appears to be a nutrients source and has a considerably high nutritive value for livestock (Balaji *et al.,* 2009). *Azolla* is considered to be the roughly promising due to good nutritive value, ease of cultivation, high productivity, and overall without any adverse effects animal health and productivity (Prabina and Kumar, 2010 ; Joysowal *et al.,* 2018). Many factors could be considered to have a major role for maintaining the productivity of the livestock animals, of which healthy gut is the major area of study in present time (Pillai, *et al.,* 2002).

When any defect in gut health is reflected on digestion and nutrient absorption, the gut consider an important and complex area involving, nutrition, immunology, microbiology and physiology, further, the gut is composed of multitude of community of microorganisms like bacteria, protozoa, fungi and viruses, while the bacteria is predominant microorganisms (Balaji, *et al.,* 2009).

The chemical composition and substrate preferences can be determined the colonization of bacteria in the gut contents (Apajalahtiv *et al.,* 2004). There is a diversity in the microorganism colonies inhabiting the gut from the proximal to the distal end with increased density in the distal part, among the portions of the Gastro- Intestinal tract (Richards *et al.,* 2005). Many factors like periods of high challenge, feed, biosecurity, environment, brooding

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conditions are influenced on the microorganism diversity and the balance of microbes in the gut (Reynolds *et al.*, 2013 and Richard, 2013). The most common infections which cause enteric diseases include adenovirus, rotavirus and reovirus (McNulty, 2003; Pierson and Fitzgerald, 2003 and Rosenberger, 2003). However, the bacterial infections the common, enteric diseases are enteritis, necrotic enteritis and ulcerative and include other diseases such mycobacteriosis, salmonellosis and colibacillosis. These pose a serious infections threat to the livability, of the livestock, as it impairs, the feeding efficiency (Khatun, 2020). So this study was designed to evaluate the effect of dietary *Azolla* To combat these enteric microorganism and to improve the gut health.

Materials and Methods

Animals and diets

This experimental work was carried out from November 2019 till 30/January, 2020 at the animal house of College Veterinary, University of Baghdad. Total animals, twenty local male rabbits, aged 4.5 - 5 month and weight range 1.526 – 1.609 kg, The animals were divided into four equal groups. Groups 1 (G1) received basal concentrate diet as the control. Group 2 (G2) received basal concentrate diet contain 5% *Azolla pinnata*, Group 3 (G3) received basal concentrate diet contain 10% *Azolla pinnata*, Group 4(G4) received basal concentrate diet contain 15% *Azolla pinnata*, and each group had water *ad libitum*. The concentrate diet was offered twice daily. The room in which rabbits were housed was hygienically maintained. Before the start of the experiment, the animal house was fumigated and all the cages were sterilized. The feeders and water bowls were cleaned daily. Consideration a basal diet according to NRC (2007) dietary ingredient (Table 1).

Estimate the faecal total bacterial number and Coliform number

Fecal samples from the experimental rabbits were collected aseptically in the end of experimental period. The samples after collection were suspended immediately in 9 mL of sterile normal saline and serially diluted from test tube 1 to test tube 7 and discarded 1 mL from test tube 7. The samples were subjected for estimation of fecal total bacterial number, fecal coliform number.

Estimate the faecal total bacterial count

Bacterial culture media was prepared by suspending 23.5 g in 1 liter of distilled water. This was brought by boil to dissolve completely and then sterilized by autoclaving for 15 minutes at 121ºC, after cooling to about 55ºC; it was poured into the petri dish and checked for sterility by overnight incubation. Then the diluted fecal sample (100µL) was spread on the plates. The plate inoculated was then incubated at 37 ºC for 24 hours as per the standard method. The colony counter for the colonies on the plate were counted used (AOAO, 1990; Sakthi Priya, 2017).

Estimation of the faecal coliform count

Mac Conkey media agar was set up by suspending 55.07g in 1 liter of distilled water. This was brought by boil to dissolve completely and then sterilized by autoclaving at 121ºC for 15 minutes. After cooling to about 55 ºC, it was poured into the petri dish and by overnight incubation to check for sterility. Then the diluted fecal sample (100µL) was spread on the plates. The cultured plate was then incubated at 37 ºC for 24 hours as per the standard method. The colony counter for the colonies on the plate were counted used (AOAO, 1990 ; Sakthi Priya, 2017).

Statistical analysis

The collected data from different parameters were pattern to Duncan's test as per the method suggested by Snedecor and Cochran (SAS.2010).

Results and Discussion

The present work involving, the *Azolla pinnata* in different levels of feeding in local rabbits at 4-5 months of age, was carried out in field conditions to investigate the phytobiotic approach in growth performance. The parameters estimated were fecal total bacterial count and fecal coliform count.

Table 1: The composition of feed ingredients used in experiment on dry matters.

Groups / Ingredient (%)	(G1) control	(G2)	(G3)	(G4)
Soy bean meal	20	15	10	5
Azolla	-	5	10	15
Corn	10	10	10	10
Barley	10	9.75	9.50	9
Wheat	25	25	25	25
Alfalfa hay	31	31.25	31.5	32
Ca	2	2	2	2
Premix	1	1	1	1
NaCl	0.4	0.4	0.4	0.4
Antitoxin	0.1	0.1	0.1	0.1
Calculated composition				
ME(kcal.kg)	13.6	13.7	13.7	13.6
CP (%)	18	18	18	18

Metabolic Energy MG/kg=
CP×0.012 +EE× 0.031+CF×0.005+NFE×0.014 (Maff, 1975).

Effect of eating *Azolla pinnata* the faecal total bacterial count

The effect of the *Azolla pinnata* on the faecal total bacterial count (Cfu/mL) is presented in the table 2. The total fecal bacterial count (Cfu/mL) after 4 weeks, post treatment was between (10^3 × 52-$10^3$63) Cfu/mL, the control group recorded the highest value as compared with the treated groups insignificantly. On the other hand the treated groups showed less different between them. While the fourth group was recorded lowest value as compared with second and third groups respectively. (10^3-$10^3$56). In control group the total fecal bacterial load was increased which was fed basal diet only then the other treated groups.

Table 2: The fecal total bacterial count (Mean±SE).

Treatments / Groups	No. of samples	Number of total bacteria	Log. Number cfu/gr /gr.(10^3)
G 1 (Control)	4	63	4.80±0.16
G 2	4	56	4.75±0.14
G3	4	61	4.78±0.10
G 4	4	52	4.72±0.09
LSD			0.127

Means in the same column are non-significantly different (P<0.05).

Effect of eating *Azolla pinnata* on the faecal coliform bacterial count

Table 3 showed the effect of the *Azolla pinnata* on the fecal coliform bacteria count (Cfu/mL), and Figure 2. The average coliform bacterial (Cfu/mL) after 4 weeks, post treatment was between ($10^3$15 -$10^3$39) Cfu/mL, the control group recorded face percentages as compared with the treated groups insignificantly, nevertheless the treated groups locked less different between them. However, the fourth group was recorded lowest value as compared with second and third groups respectively (10^3 35- $10^3$28) in the fecal coliform bacterial load.

Table 3: The faecal coliform bacterial count (Mean±SE).

Treatments / Groups	No. of samples	Number of coliform bacteria /gr.(10^3)	Log. Number cfu/gr
G1 (Control)	4	39	4.59±0.12
G 2	4	35	4.54±0.14
G 3	4	28	4.45±0.15
G4	4	15	4.18±0.14
LSD			0.462

Means in the same column are non-significantly different (P<0.05).

Means in the same column are non-significantly different (P<0.05)

Many researchers refer to the total fecal bacterial count and faecal coli from count reduction in the herbal preparation fed animals may be attributed to the herbal in the diet eating. The flavonoids present in *Allium sativum* exhibits helped bactericidal and bacteriostatic and effects on some strains of bacteria by discouragement proteases activity and reverse transcriptase in bacteria (Gazuwa *et al.,* 2013). Whilst, the garlic exerts have a differential inhibition between intestinal microflora beneficial and enterobacteria potentially harmful, like observed inhibition in *E. coli* was more than ten times greater than that seen in *Lactobacillus casei* (Cardelle *et al*., 2010).

Gingerols effect on increase the gastrointestinal tract motility and have antipyretic, analgesic, sedative, and antibacterial properties in laboratory (Sharma *et al.,* 2006). Also the *Zingiber officinale* contain antimicrobial property against have adverse effect on harmful microorganism *Pseudomonas aeruginosa, E. coli, Enterococcus faecalis, Staphylococcus aureus* (Bhargava *et al.,* 2012). However, the some researchers declared the 0.5% level of herbal in diet optimum level for improve the gut health and thereby reducing the enteric pathogens. Allinson (Allinson *et al.,* 2013; Ghalib. 2010). Important source for antibacterial components, proved that the fenugreek was found to strongly inhibit the *Staphylococcus* growth (Basu *et al.,* 2009). From the above, there could be a disincentive effect to the inner microorganisms when using *Azolla*. That confirms with the results obtained by Nayak *et al.,* (2015). *Azolla* can be used as an antibacterial and antioxidant agent in complementary and alternate medicine, had been recommended due to its raise flavonoid and phenolic content (Noor *et al.,* 2014). A part from *Azolla* nutrients, also contains certain compounds such as, bio-polymers, carotenoids and probiotics which contribute to above productivity and healthy of animals (Parashuramulu, and Nagalakshmi, 2012).

The result from this work indicated that dietary adding of *Azolla pinnata* up to a level of 15% causes a definite reduction in the fecal total bacterial count and fecal coliform count. Furthermore studies are needed to assess the effect of the *Azolla* on production performance of animals.

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