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An Entire Process for the Isolation of Blood Meal from Animal Blood & Microbial Investigation in Blood Meal

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***Abstract**: Blood is rich source of iron and proteins of high nutritional and functional quality. Cattle are slaughtered enormously all over the world every day. But in maximum cases this blood is discharged in sewer, thus a valuable protein source is wasted as well as resulting in serious environmental pollution problems. Best utilization of blood in calculative ways can save the environment from such virulent pollution. This study intended to isolate the blood meal from blood through a conventional way and analyze different microorganism through their morphological characteristics, size, shape, before and after radiation. The microbial investigation has been carried out in three categories Total Bacterial Count (TBC), Total Coli form Count (TCC) & Total Fungi Count (TFC). After drying 5×108 cfu/gm TBC and 1.7×10 7cfu/gm TCC were found while no TFC was found in the agar plates. Finally Gamma radiations with different dozes were implemented to make it useable and 63 % protein was isolated. This valuable protein is edible and could be used in different areas.*

***Keywords:** Blood, Blood Meal, Environmental Pollution, Isolation of Blood Protein, Micro-Organism,*

I. Introduction

Blood is regarded as a high valuable proteins source, about 18% protein contains in the blood [1]. Because of the high protein content, sometimes it is referred as "liquid protein"[2]. Hundreds of millions of animals are slaughtered each year all over the world. Thus huge amount of blood are generated every day, where only a small percentage is used in different areas while maximum are wasted. The total weight of blood from the domestic animals is equivalent to 6 to 7% of the lean meat in the carcass, whereas the whole quantity never completely recovered from the animal body [3, 5]. Of all waste products, the waste in the form of blood has the highest polluting value. Blood itself has a high BOD: 150,000 - 200,000 mg/l, the extreme value is 405,000 mg/l [4]. Therefore, when the blood is produced at the time of killing, bleeding and skinning phases, if it is disposed in sewer completely, as waste which pollutes the canals and rivers water resulting aquatic life faces threats causes of decreasing the dissolved oxygen level in the water [4]. So, to get rid of a considerable pollution hazard and prevent from the loss of a valuable protein source, efforts have been made to ensure the proper utilization of animal blood on a massive scale.

The objective of this work was to isolate the blood proteins for using as a protein supplement in the animal feed. Furthermore a microbial investigation was performed to find out the morphological phenomenon of different micro organisms and their activities. As blood is clotted very quickly by the action of thrombin and calcium on fibrinogen, which present in the plasma and converted into insoluble fibrin, so blood collection is the prime and arduous operation in here [5]. Therefore, anticoagulant must be used to prevent clots in container before blood was collected. As this blood is protein, it contains high microbial load [6]. So that microbial investigation were required to find out micro organisms and their characteristics in the meantime a strong sterile method was required to eradicate such pathogens. Consequently gamma radiation was implemented to sterile, which is the most suitable method in here than any other method. Unlike heat sterilization or steam sterilization, gamma irradiation has no significant effect on the physical and chemical properties of the yielding product [9].

The blood meal contains important protein fraction. The amino acid profile of that protein fraction is very similar to the ideal protein ratio, where it lysine content is very high comparatively with other food protein, like bone meal, meat meal [7,8]. This protein could be used as an alternative protein source as a foodstuff for animal feed, poultry feed and other species, where protein is an important factor. Efficient utilization of such by-products has direct impact on the economy and environmental pollution [11]. So maximum utilization of blood in proper way must be ensured, otherwise wasted animal blood may create major unhygienic and catastrophic ecological problems.

II. Materials and method

2.1 Blood collection:

Fresh blood was collected from a slaughter house simply immediate after the slaughtering. Care had taken, so that only clean blood be collected, where contamination with undigested food from the stomach can be prevented from grasping the esophagus firmly at the moment of slaughtering [5] .To prevent clots of blood 0.2% citric acid was used as anticoagulant agent, that was diluted with 2 part water and must be kept in the container before blood was collected [5].

2.2. Centrifugation

Blood consists of 60% plasma and 40% cells in volume also contain urine, ammonia and other undesirable ingredient along with protein [7]. To separate the blood cell from plasma, blood was centrifuged in the centrifugal machine at 3000 rev/min with the time 15 minutes. Then the blood cell was collected from plasma.

2.3. Drying:

Drying was an inevitable operation to dwindle the moisture level. The separated blood cell was desiccated in a vacuum drier, where vacuum was created by the vacuum suction pump & regulated air pressure was adjusted 0.06 Mpa. Initially the sample was dried at 50˚C for 3hours then examined by moisture analyzer and 39.36% moisture was found. Then temperature of the drier was adjusted at 60˚C and dried for 1.5hours to get the resultant moisture.

2.4. Culture Preparation:

Bacterial strains inside the blood were cultured in solid Nutrient Agar media. NA media composition was as following: 0.5% peptone, 0.3% beef extract, 1.5% agar, 0.5% NaCl. pH was adjusted to 6.8.

2.5. Irradiation:

Irradiation was the most important step to remove pathogens that were grown in the blood meal. Electromagnetic gamma radiation, which was generated by the using of the Cobalt 60's isotope and this electromagnetic radiation were performed as irradiation with different level of dozes 2.5 KGy,5 KGy,7.5 KGy,10 KGy after packaging of the products and was involved any aseptic handling. The gamma radiation of cobalt 60 was produced from Institute of Food and Radiation Biology, Atomic Energy Research Establishment, Saver, Dhaka, Bangladesh.

2.6. Food ingredients analysis:

2.6.1. Protein Contains: Total protein contain in the blood meal was estimated by Kjeldhal method which is the internationally recognized method for estimating the protein content in foods. The estimation was done just immediate after radiation without any ambience contact.

2.6.2. Fat Contains: Percentage of fat was analyzed by Soxhlet apparatus official method of analysis of AOAC INTERNATIONAL, Method 960.39 and 948.22 18th editions AOAC INTERNATIONAL, Gaithersburg, MD (2005).

2.6.3. Ash Contains: Ash contain was determined by AOAC official method of ash in animal feed 942.05 [12].

III. Results & Discussions

Blood meal is hygroscopic which axiom that, control of moisture is mandatory and less than 10-12 % moisture is allowed to prevent it from deterioration [8]. The final moisture of the product was recorded 10.17% (Table: 1).

TBC (Total Bacterial Count), TCC (Total Coli form Count), TFC(Total Fungi Count) were three different categories that microbial investigation were maneuvered. Blood meal Protein is suitable enough for proliferation of bacteria and fungi (Table 2), which signify that huge amount of TBC and Coli form were grown but in result no TFC were found. The isolated bacteria in most occasions were aerobic and facultative anaerobic. In the present study the organism isolated were staphylococcus, diplococcus, rod shaped Bacilli and cornybacteriam, micro-coccus (Table3). Morphological test were done to confirm the strain.

TBC were cultivated in nutrient agar, as a result staphylococcus, diplococcus and bacillus were found with different in sizes, shapes, colors and elevations (Table 3). Analyzing Microscopic images, the amounts of staphylococcus and bacillus were (Table 4& Table 6) shown a positive result before and after irradiation. Perusing the microscopic images (Table 4) it was found that, staphylococcus and bacillus were prolific amount at earlier stage but it was reduced at a great extend after irradiation with 2.5 KGY(Table 6).

MacConkay agar was used to find *E. coli* if there were any but nothing was found rather than corny bacterium, coccus and micrococcus were developed (Table 3).

The pathogens staphylococcus can cause a wide range of diseases in human through either toxin production or penetration. Any organ of human being especially skin can be affected by several species of staphylococcus particularly *Staphylococcus aureus* and *Staphylococcus epidermidis* [11]. *Staphylococcus aureus* pathogen is also responsible for bumble foot disease which is common infection domesticated in poultry and waterfowl such as ducks and chickens. This chronic inflammatory disease is responsible for significant economic loss in commercial poultry operation [13]. Diplococcus, *Streptococcus Pneumoniea* is responsible for bacterial meningitis [14].

However different Bacillus species like as *Bacillus cereus, Bacillus anthracis* are also cause serious lethal activities to human and animal. *Bacillus cereus* associated mainly with food poisoning, also responsible for serious and potentially fatal non-gastrointestinal tracts infections [15]. *Bacillus anthracis* spores cause natural infections, which are etiological agent of anthrax, a common disease of livestock and occasionally of human [16, 17]. On the other way cornybacterium are mostly innocuous [18]. But there is an exception, *Corynebacterium diphtheria* which pathogen is responsible for diphtheria [19]. Generally Species of micrococci are considered as non-pathogenic commensals however, some species are opportunistic pathogens for the immune-compromised. *Micrococcus luteus* has been reported as the causative agent in cases of intracranial abscesses, pneumonia, septic arthritis, endocarditic, and meningitis [20].

So, irradiation was implemented with different dozes to remove such pathogens, but at 2.5KGY (Table 5) only trivial amount TBC were survived .Therefore, 5 KGY radiations is adequate to eradicate all kinds of organisms. The final product was dark brown in color and odorless with pH 7.89 [6]. By following oxitop method the BOD value of the final product was found 1600 m/l (Table 7) which was comparatively very lower than the BOD value 150,000-200000 mg/l of raw blood. Approximately 63% protein, small amount of fat and ash (Table 8) were isolated by this process. Unlike other animal protein sources, blood meal is easily digestible and has coefficient amino acid balance [21]. Therefore it could be used as cattle, poultry, fish & other species food as protein supplement. In steers and calves blood meal inclusion in dietary could assist them to increase daily weight grain [22]. Furthermore in dairy cows it may improve both the milk production and protein [23]. So, partial sharing with other protein ingredient in feed ensuring improvement of performance, greater profit and to save the environment from massive pollution.

Table-1: Moisture Analysis.

Observation No.	Duration (hrs)	Temperature (˚C)	Pressure (Mpa)	Result (%)
1	3	50	0.06	39.36
2	1.5	60	0.06	10.17

Table:2 Colonial Count Of TBC,TCC,TFC.

TBC(cfu/g)	TCC(cfu/g)	TFC(cfu/g)
5.0×10^8	1.7×10^7	Nil

Table:3 Colony characterization of TBC & TCC isolated from blood meal before irradiation

	Organism	Color	Size	Form	Elevation	Opacity
TBC	Staphylococcus	White	Small	Circular	Raised	Opaque
	Diplococcus	Brownish	Big	Round	Down	Translucent
	Staphylococcus	Yellowish	Big	Round	Concave	Opaque
	Staphylococcus	White	Big	Round regular	Concave	Opaque
	Bacilli	white	Big	Irregular	Flat	Opaque
TCC	Cornybacterium	Reddish/Pinkish	Small	Round	Convex	Opaque
	Micrococcus	Pinkish	Big	Round &regular	Centrally concentrated	Opaque
	Coccus	Whitish	Small	Irregular	Smooth	Opaque
	Coccus	Pinkish	Big	Regular	Centrally concentrated	Opaque

Table-4: Colony characterization and microscopic image of TBC & TCC isolated from blood meal before irradiation.

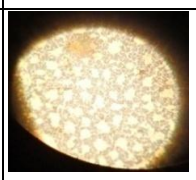
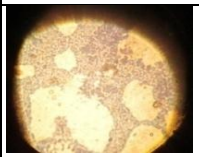
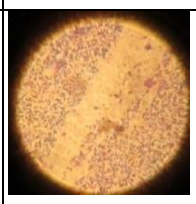
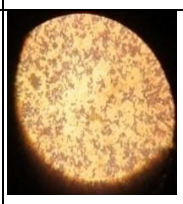
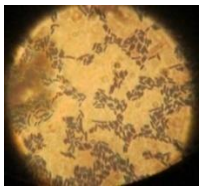
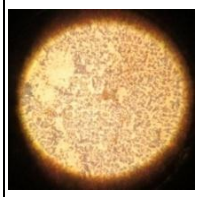
TBC				TCC			
Organisms	**Gram staining**	**Arrangement**	**Microscopic images**	**Organisms**	**Gram Staining**	**Arrangement**	**Microscopic images**
Staphylococcus	(+) cocci	Cluster forming		Corny-bacterium	(+) cocci	Cluster forming	
Diplococus	(+) cocci	Cluster forming		Micro-coccus	(+) cocci	Cluster forming	
Bacilli	(+) Rod	Cluster forming		Coccus	(+) cocci	Cluster forming	

Table: 5 Colonial count after irradiation

Radiation(KGy)	TBC(cfu/g)	TCC(cfu/g)
2.5	2×10^3	Nil
5	Nil	Nil
7.5	Nil	Nil
10	Nil	Nil

Table-6: Morphological characterization & microscopic images of survival bacteria isolated from blood meal after irradiation

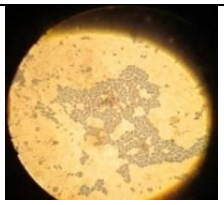
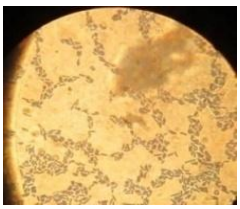
Organism	**Color**	**Size**	**Shape**	**Opacity**	**Gram's staining**	**Arrangement**	**Microscopic image**
Staphylococcus	Whitish	Medium	Round & regular	Opaque	(+) Cocci	Cluster forming	
Bacilli	Whitish	Big	Round & regular	Opaque	(+) Rod	Cluster forming	

Table: 7 Physical & Chemical properties of yielding products

Physical properties		Chemical Properties	
Color	Dark brown	**pH**	**7.89**
Odor	odorless	**Solubility**	In soluble in acid &alkali
Physical State	Aggregate form	**BOD_5(Biochemical Oxygen Demand)mg/L**	1600

Table 8: Percentages of food ingredients

Protein	63%
Fat/Oil	2%
Ash	4-5%
Moisture	10.17%

IV. Conclusion

Environmental pollution problem is a major concerning factor now this time. The production of protein is profitable because of availability of raw material and requiring a few common equipments to extract blood meal. If this protein can be used in poultry feed, animal feed or other sector as a protein supplement, it will be beneficial for economy and environment.

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[1]. Putnam FW, The plasma proteins: structure, function, and genetic control, (New York: Academic press,1975).
[2]. Ockerman HW, Hansen CL. Animal by-product processing and utilization, (L.T.P. comp. Inc. 2000) 325-353.
[3]. Wismer-Pedersen J, Use of hemoglobin in foods-A review, Journal of Meat Science, 2(1), 1988, 31– 45.
[4]. Verheijen LAHM, Wiersema D, Hulshoff Pol LW, Wit JD, Management of Waste from Animal Product Processing, International Agriculture Center, Wageninge ,Netherlands, 1996.
[5]. MANN, Utilization of blood, Process & utilization animal by-products, animal Industry Projects Sec., Department of Veterinary Services, Ministry of Agriculture, Animal Husbandry and Water Resources, Kabete, Kenya, Ed 4th, 60-63.
[6]. Zieglar J, Dried blood pigment preparation for comminuted meat products and method of preparing same, patent no :Us 3073700 A, January 15 ,1963.
[7]. Gatnau R, Polo J, Robert E, Plasma protein antimicrobial substitution at negligible risk, In :Bru fau J.(ed.), Feed manufacturing in the Mediterranean region. Improving safety: from feed to food. Zaragoza : CIHEAM.141 -150 (Cahiers Options Méditerranéennes; n. 54, 2001.
[8]. Batterham ES, Lowe RF, Darnell RE, Major EJ, Availability of lysine in meat meal, meat and bone meal and blood meal as determined by the slope-ratio assay with growing pigs, rats and chicks and by chemical techniques, British Journal of Nutrition, 55(02), 1986, 427-440.
[9]. Gharaghani H, Zaghari M,Shahhosseini G, Moravei H, Effect of Gamma irradiation on anti nutritional factors and nutritional value of canola meal for broiler chickens, Asian-Australian Journal of Animal Science, October 1, 2008.
[10]. Jayathilakan K , Khudsia S, Radhakrishna k, Bawa AS, Utilization of by products and waste materials from meat, poultry and fish processing industries, Journal of Food Science and Technology, 49(3), 2012, 278-293.
[11]. Rosanna C,Josephine M, Malcolm JH, Staphylococci: colonizers and pathogens of human skin, Future Microbiology, 9(1), 2014, 75-91.
[12]. Thiex N, Novotny L, Crawford A(2012). Determination of Ash in Animal Feed: AOAC Official Method 942.05 Revisited. J. AOAC. Inter.95(6): 1392-1397.
[13]. Wilcox CS, Patterson J , Cheng HW, Use of thermography to screen for subclinical bumblefoot in poultry, Poultry of Science, 88 (6), 2009,1176-1180.
[14]. Alonso De VE, Verheul AF, Verhoef J, Snippe H, Streptococcus pneumonia, virulence factors, pathogenesis, and vaccines: Microbiological Review, 59(4), 1995, 591-603.
[15]. Edward JB, Bacillus cereus, a Volatile Human Pathogen, Clinical microbiology review, 23(2): 2010, 382-398.
[16]. Logan NA, Bacillus species of medical and veterinary importance, Journal of Medical Microbiology, 25, 1988, 157-165.
[17]. Mayer-Scholl A, Hurwitz R, Brinkmann V, Schmid M, Jungblut P, et al. () Human neutrophils kill Bacillus anthracis. PLoS Pathog 1(3): e23, 2005.
[18]. Matthew DC, Lesley H, Geoffrey F, Enevold FCorynebacterium caspium sp. nov. from a Caspian seal Phoca caspica, Internation Journal of System Evolution Microbiology 54 (3) (2004), 925-928.
[19]. [Ted LH, Peter MacEvoy,Yury P, Vesevolod AT, Alexey AY, The pathology of diphtheria, Journal of Infection Disease 181(1), 2000, 116-120.
[20]. Bannerman TL, Peacock SJ, Staphylococcus, Micrococcus, and Other Catalase- Positive Cocci. In Murray PR, Baron EJ, Jorgensen JH, Landry ML, Pfaller MA(Eds.), Manual Clinical Microbiology 9, (Washington, USA: ASM Press 2007) 390-404.
[21]. Ravindran V, Hew LI, Ravindran G , Bryden WL, Apparent ideal digestibility of amino acids in dietary ingredients for broiler chickens, Cambridge Journal Online, 81(1), 2005, 85-97.
[22]. Knaus WF, Beermann DH ,Robinson TF, Fox DG, Finne KD, Effects of a dietary mixture of meat and bone meal, feather meal, blood meal, and fish meal on nitrogen utilization in finishing Holstein steer, Journal of Animal Science, 76(5) , 1998,1481-1487.
[23]. Paz HA ,Kanonoff PJ, Lactation responses and amino acid utilization of dairy cows fed low-fat distillers dried grains with soluble with or without rumen-protected lysine supplementation, Journal of Dairy Science, Published Online, August 06, 2014