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Editorial Message

Dear Doctor,

It gives me immense pleasure to wish you Happy New Year 2022 to all our readers/members and veterinary professionals.

The livestock sector in India plays a major contributor to the agricultural economy of our country, not merely in terms of income but also in terms of livelihood and employment. It is usually said that livestock wealth is more equitably distributed than agricultural land. There is an upwards flow of demand for livestock products due to increase in population, growing per capita income and better living standards.

A large number of farmers in India depend on animal husbandry for their livelihood. In addition to supplying milk, meat, eggs, wool and hides animals i.e. cattle, buffalo, sheep, goat, pig etc. are the major source of power for farmers. Thus animal husbandry plays an important role in the rural economy. In this context 'Vets Review' plays as a precursor for the upliftment of animal husbandry sector as well as helps veterinarians to disseminate their scientific knowledge for better management of animal health. We think this is an opportunity for us to renew its usefulness and the value that it will try to provide our readers. We therefore encourage you to write to us with suggestions on how to make it better.

On this hopeful note, we were in our happy journey and let us try to convert it to a National Journal.

Editors

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MESSAGE

I am very happy to learn that the 8th Annual General Meeting of the Progressive Veterinary Doctors' Association will be held on 27th March, 2022 at West Bengal Veterinary Council Auditorium, Belgachia, Kolkata. It is also learnt that a large number of Veterinary Doctors/Scientists and other dignitaries will congregate during this occasion.

I hope that this occasion will throw up opportunities not only for discussing the developments achieved in the field of Veterinary Sciences but also for seeking ways and means to tackle the problems faced by the Veterinary practitioners during their field work.

I am also happy to learn that to commemorate the occasion, the Association is going to bring out the 7th Edition of the Scientific Magazine "Vets Review" which will highlight the important scientific issues viz. sustainable parasitic control, feeding management of goat, bypass protein technology for improvement of livestock production and rearing of ornamental birds etc. I hope that the edition of the Magazine will be informative and helpful for the Veterinary Professionals.

I wish all the best for the success of the 8th Annual General Meeting of the Progressive Veterinary Doctors' Association.

Chanchal Guha
(CHANCHAL GUHA) 11/03/2022

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PREFACE

This is our great pleasure to inform you that scientific wing of Progressive Veterinary Doctors' Association with their almost venture and enthusiasm could finally publish the 7th Edition of Vets Review.

Progressive Veterinary Doctors' Association from the very beginning of its formation, takes pledge to work for the upliftment of Scientific Mindset of our fraternity. We strongly believe that our bulletin is a good platform for exchange of scientific thoughts on animal production and animal health among veterinarians holding different responsible chairs including laboratories, dispensaries, administration, teaching, research and development and extension activities.

Therefore, on the auspicious occasion of 8th Annual General Meeting of PVDA held on 27th March, 2022 at West Bengal Veterinary Council Hall, Belgachia, Kolkata we are going to publish the 7th Edition of our technical bulletin. The current issue is enriched with over view of Fatty Acid composition of meat and its importance for human health : special emphasis on CLA enhancement in Goat Meat, Bypass Protein Technology for improvement of Livestock, Productivity in Ruminants, Ornamental Bird Rearing : Alternative Source of Income Generation, Effective Parasite Control–a Futuristic Approach, Evaluation of Different Methods for Viral DNA Extraction from, Sheep Pox Virus Infected Cell Culture, Pseudomalaria in Domestic Pigeons : A Case Report, Incidence of Canine Babesiosis and Ehrlichiosis in and around Chinsura, Hooghly, West Bengal, A Retrospective Study on Cutaneous Hemangiosarcoma in 21 Dogs, Eimeriosis in Cattle & Haemato-biochemical changes : A Case Report, A Natural Alternative to Present-day Anthelmintic Drugs : Cysteine Proteinases with a Special Reference to Gastrointestinal Nematodes, Behavior Management in Dog, Present Status of Female Cattle and Milk Production in West Bengal, Different Treatment Scheduled in Anovulatory and Delayed Ovilatory Indigenous Cows of West Bengal.

I would like to thank our scientific magazine committee who has taken such good effort to publish this edition in time. We hope that authors, colleagues, and readers could appreciate all the time and effort expended in preparing this issue.

With thanks

(Dr. Chayan Bhattacharya)
General Secretary
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FATTY ACID COMPOSITION OF MEAT AND ITS IMPORTANCE FOR HUMAN HEALTH : SPECIAL EMPHASIS ON CLA ENHANCEMENT IN GOAT MEAT

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Introduction

The prospects for improving the fatty acid profile of milk and meat from ruminant animals represent a growing market for the global livestock sector as a means to support better human health. Food products obtained from ruminant animals are important source of nutrients, supplying high quality protein, energy and a variety of minerals and vitamins (Mills *et al.*, 2011). The greater proportion of saturated fatty acids (SFA) in ruminant meats compared with other protein sources has become a subject of concern because of the potential role of dietary SFA in the etiology of obesity, hypertension, and coronary heart disease in humans (Givens, 2005). Besides a lower total fat intake, human nutritionists are recommending a higher intake of unsaturated fatty acids because of their health-promoting effects (Raes *et al.*, 2004). Among these fatty acids, conjugated linoleic acid (CLA) and vaccenic acid (VA), including other 18:1 monoenes, are the microbial metabolites from rumen. Recent studies have demonstrated potential benefits of CLA for human health through putative anticarcinogenic, antioxidative, and antidiabetic effects (Elwood *et al.*, 2010). Therefore, modifying ruminal microbial metabolism of fatty acid in rumen through animal diet formulation is an effective way to improve the fatty acid composition of ruminant-derived foods, such as milk or meat, and their products to reduce saturated: unsaturated fatty acid ratio and increase CLA content may have value to the milk and meat industries (Radunz et al., 2009; Or-Rashid *et al.*, 2009).

The fat content of meat

Fat is an essential component of meat for sensory perception of juiciness, flavor, and texture. Fat in meat also supplies fatty acids that cannot be synthesized by humans. The perception of healthiness and sensory expectation are important quality criteria that influence the decision of a consumer to purchase a particular food product. Fresh meat production systems represent the combined and interacting effects of genotype, gender, age at slaughter and nutrition before slaughter, all of which can contribute to differences in the fat concentrations of fresh meat.

The opportunities to exploit the diet of meat animals to produce flavoursome meat that has an increased concentration of CLA, a compound that may protect against obesity, cancer and heart disease, a low fat concentration (25–50 g/kg) in modern lean red meat and can be considered as a low fat food and a fatty acid profiles more compatible with current human dietary recommendations will be illustrated including the possibility of meat being recognized as a functional food.

Fatness

Fat in meat can be present as intermuscular fat (between the muscles). Intramuscular fat (or marbling, i.e., within the muscles) and subcutaneous fat (under the skin). Most of the fat is present as glycerol esters but cholesterol, phospholipids and fatty acid esters are also present (Jimenez-Colmenero et al., 2001). Due to growing consumer preferences for low fat food products, the red meat industry began in the early 1980s to modify production systems to produce less fat in meat. The fat content of the carcass has decreased in Britain by over 30% for pork 15% for beef and 10% for lamb through selective breeding and feeding practices designed to increase the lean to fat ratio; official carcass classification systems designed to favour leaner production; and modern butchery techniques (seaming out whole muscle and trimming away all intermuscular fat) (Higgs, 2000).

While the meat industry continues to address consumer preferences for lower fat meat and meat products, the relationship between the fat content and sensory perception must be considered. In some meat production systems (USA), high intramuscular fat content (marbling) has been associated with superior tenderness, juiciness and overall satisfaction. Moreover, many of the flavor compounds of meat are contained in the fat component or are released due to chemical changes in the fat, alone and in interaction with the protein component, during ageing and cooking. The consensus of opinion now is that a decrease in intramuscular fat content to 2-5% with a relatively greater reduction in 'waste' fat depots or

altering the fatty acid compositions of meat would make a positive contribution to production efficiency and consumer health without negative impacting on meat quality.

Fatty acid composition of meat

Overall, lean red meat contains similar proportions of MUFAs to SFAs, although, the exact proportions may vary depending on the type of meat and proportions of lean and fat. The main SFAs present in red meat are palmitic acid and stearic acid (MAFF, 1998). There are also minor amounts of myristic acid which is thought to increase cholesterol levels more potently than palmitic acid, while stearic acid appears to have no effect on cholesterol levels, despite being a SFA (Higgs, 2000). The principle MUFA in meat is oleic acid, and typically between 30 - 40 % of the fat in meat is composed of MUFAs. Red meat also contains PUFAs (8-15%) predominantly linoleic (n-6) and α- linolenic acid (n-3), which are known as essential fatty acids. Although meat contains low levels of PUFAs, in UK, meat and meat products contribute substantially to intakes, providing 18% of n-6 PUFAs and 17% of n-3 PUFAs, while contributing to 23% of overall fat intake (Talpur et al., 2008). In Denmark, meat and meat products contribute 26% of MUFAs, 11% of PUFAs and only 18% of SFAs in the diet (Danish Institute for Food and Veterinary Research, 2005).

Meat also contains small amounts of the long chain n-3 PUFAs eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA). Studies have demonstrated that long chain n-3 PUFAs, e.g. EPA and DHA, have potential benefits in relation to heart health, especially in those who have already had a heart attack. The UK Department of Health recommends that intake of long chain n-3 PUFAs should be at least 450mg per day (SCAN & COT, 2004). Meat from ruminant animals is also a source of naturally occurring fatty acid CLA (2-6 mg/ g of fat) which has several health beneficial effects.

Current health trends and ruminant products Saturated fatty acids and cholesterol

Generally, SFA are the most abundant FA in the human diet owing in part to the SFA content of vegetable oils used in cooking and processed foods (Ailhaud et al., 2006). Increased globalization has led to cheap foreign processed products now competing with local foods, which has altered traditional diets. This has led to a concomitant increase in obesity, cardiovascular disease (CVD) and other metabolic disorders coined “diseases of the developed world” emerging in the developing world. The greatest health risk to humans posed by dietary SFA is by increasing the plasma total cholesterol and low-density lipoprotein (LDL) levels. High LDL and total cholesterol levels are known risk factors for the development of CVD (Lefevre et al., 2004). The increased risk comes from oxidized LDL becoming adhered to the arterial epithelial layer leading to the accumulation of cholesterol esters and formation of plaque deposits. High density lipoproteins (HDL) play a crucial role in reducing these arterial deposits by absorbing the cholesterol esters and transporting them back to the liver. Studies generally have implicated C12:0, C14:0 and C16:0 in elevating total cholesterol and LDL levels (Williams, 2000), whereas, C18:0 has shown neutral or slightly beneficial effects on cholesterol levels and associated CVD development (German and Dillard, 2004).

Health effects of cis-and trans-onounsatura ted fatty acid

Compared to plasma LDL raising SFA, cis-MUFA are considered to be more beneficial for human health by reducing inflammation and blood coagulation factors with no associated reduction to plasma HDL levels. Alternatively, dietary trans-FA act like SFA by raising plasma LDL levels; however trans-MUFA are considered to be more potent than SFA due to associated reductions to the plasma HDL level (Williamson et al., 2005). Trans-MUFA found in commercial vegetable oils are formed during the refining process and have been shown to reduce plasma HDL level, whereas trans-MUFA from ruminant origin have not shown to alter plasma HDL levels (Williams, 2000).

Conjugated linoleic acid and associated health benefits

A number of beneficial physiological effects have been attributed to CLA c-9, t-11 and CLA t-10, c-12 including anti-carcinogenic and anti-obesity effects, as well as lowering the risk of developing atherosclerosis by lowering plasma LDL levels (Tyagi et al., 2011). Whereas supplementing CLA in animal trials has shown positive effects, results from human studies using supplemented CLA are limited and often contradictory. Differences in trial design, subject selection, administered dose and gender have all contributed to the contradictory results.

Milk has been shown to have a higher CLA content than meat, though both are a dietary source for humans. The proportion of each major isomer in ruminant products can vary with ruminant diet. In general, the CLA c-9, t-11 isomer has been shown to constitute between 75 to 90% of the CLA found in ruminant products, with CLA t-10, c-12 accounting for an additional 1 to 2% (Lock and Bauman, 2004). Levels of CLA c-9, t-11 in ruminant products have been enhanced by forage feeding or

supplementation with oilseeds. Increased levels of CLA t-10, c-12 in ruminant products are generally associated with feeding diets containing high levels of concentrate (Chilliard et al., 2007).

Polyunsaturated fatty acids and inflammatory response

Dietary PUFA help to reduce the risk of developing CVD by raising plasma HDL levels while lowering both plasma total cholesterol and LDL levels (Wijendran and Hayes, 2004). Besides affecting cholesterol levels, dietary PUFA, particularly LC-PUFA, play an important role in producing eicosanoids, biologically active lipids that modulate the body's inflammatory response. The conversion of C18:2n-6 and C18:3n-3 to their LC-PUFA derivatives is considered to be low in mammals; hence humans rely considerably on dietary sources of LCPUFA (Chapkin, 2008). The relative proportions of tissue n-3 and n-6 LC-PUFA act as a regulator for localized eicosanoid production and activity, ultimately influencing the physiological functions of the body. The LC-PUFA are divided into two families based on their initial substrate and can be generalized as either n-6 pro-inflammatory or n-3 anti-inflammatory LCPUFA based on the eicosanoids derived from them.

Table 1: Comparisions of FA composition (g/ 100 g FA) of crossbred (Alpine x Beetal) male goat kids in different muscles and tissue

Muscles and fat →	*Longissimus dorsi*		*Semitendinosous*		*Adipose tissue*	
Fatty acids ↓	C	T	C	T	C	T
∑SFA	57.18	51.22	59.08	51.94	51.86	49.65
∑MUFA	32.20	33.41	30.99	34.24	36.84	37.63
∑PUFA	10.63	15.38	9.93	13.90	11.30	12.76
∑UFA	42.83	48.78	40.92	48.14	48.14	50.39
∑UFA/∑SFA	0.75	0.95	0.69	0.93	0.93	1.01
∑*trans* FA	2.08	2.09	3.35	3.28	1.95	2.05
∑n-6	8.33	11.24	7.51	10.36	9.41	10.35
∑n-3	2.30	4.14	2.42	3.54	1.89	2.41
∑n-6 / ∑n-3	3.62	2.72	3.10	2.93	4.99	4.30
CLA (c-9,t-11)	0.37	0.50	0.44	0.56	0.64	0.93
CLA (t-10,c-12)	0.04	0.06	0.04	0.06	0.07	0.07
∑CLA	0.40	0.57	0.49	0.62	0.70	1.00

C : Control animals fed with basal diet
T : Treatment animals administered with Butyrivibrio fibrisolvens 4a strain [GenBank Accession No.- JX867371], @ 109 CFU/head for a week directly into rumen and fed dietary oil rich in linoleic acid @ 200 µg/ml of rumen volume along with control diet.

Prostaglandins are generally considered to be pro-inflammatory (PGE2, PGF, PGD) but can also reduce blood platelet aggregation (PGE1, PGI), further blurring the line between pro-inflammatory and anti-inflammatory responses (Lee and Hwang, 2008). Thromboxanes (TXA) induce blood platelet aggregation, whereas leukotreines (LTB) elicit allergic reactions. The antagonistic actions of the n-6 series eicosanoids are generally many times higher than those derived from the n-3 series (Lands, 2008).

Antiinflammatory eicosanoids produced from C20:5n-3 include series 3 prostaglandins (PG3) and series 5 leukotrienes (LTB5). Overstimulation of the inflammatory system has been linked to a number of human chronic diseases including atherosclerosis, asthma, and a number of cardiovascular related events (Calder, 2006). Moreover, C20:4n-6 has been shown to be a potent promoter of adipogenesis, leading towards obesity (Ailhaud et al., 2006). Alternatively, studies have indicated C22:6n-3 is essential for neurological function and furthermore, exhibits strong positive cardiovascular effects. The function of C22:5n-3 has not been investigated as extensively as C20:5n-3 or C22:6n-3; however there are indications that C22:5n-3 is a more potent inhibitor of platelet aggregation than either C20:5n-3 or C22:6n-3 (Akiba et al., 2000).

Many mechanisms are involved in the modulation of the relative proportions of LC-PUFA within the membranes. The formation of n-6 LCPUFA is known to be reduced through competitive inhibition by n-3 LCPUFA. The higher affinity of desaturase enzymes towards n-3 FA has also been shown to reduce the relative production of n-6 derived eicosanoid, thereby reducing the health risks associated with a pro-inflammatory state (Lee and Hwang, 2008). Moreover, n-3 LC-PUFA have been shown to be poor substrates for eicosanoid production, thus mitigating the intensity of the inflammatory response (Lands, 2008).

Present and future scenario of meat industry and scope for goat meat

Red meat provides many essential nutrients, including fats, and can be an important part of a balanced diet (Williamson et al., 2005). Globally, there has been a general trend towards increased meat consumption, rising by more than 10% since the 1960's (Valsta et al., 2005). Today, health concerns have been raised about saturated fatty acids (SFA) as well as the amount and types of polyunsaturated fatty acids (PUFA) ingested. Consumers often have been critical of ruminant meat due to the high SFA and low PUFA/SFA ratio. Moreover, the use of concentrates in modern livestock production has led to an increase in the n-6/n-

3 PUFA ratio of ruminant meat. Cumulatively, this has led to a decline in beef sales during the last 40 years (Givens et al., 2006).

Over the past few years, meat from goat has gained acceptance around the world mainly because it is leaner than beef and mutton and has low cholesterol content (Mahgoub et al., 2002). Adrizzo (1999) has repoted fatty acid composition of goat meat with low levels of lauric, myristic and palmitic acids, which are associated with the biosyntheseis of cholesterol. Goats have also been reported to be efficient converter of low quality feed into a valuable animal protein. Regardless of the importance of goat as a source of lean meat, the nutritive value of goat meat has received little attention and consequently there are very few reports on the fatty acid composition of meat and adipose tissues from different goat breeds. These studies are mostly from young goats slaughter at litter body weights (up to 30 kg) (Mahgoup et al., 2002).

Enhancement of CLA in goat meat

Goats are an important nutrient source (meat and milk), particularly for people in the developing regions, situated mainly in the tropics. These regions account for more than 90 percent of the estimated world goat population of 504 million, with approximately 56 percent in Asia, 33 percent in Africa and 7 percent in South and Central America and the Caribbean. Chevon (goat meat) is one of the most widely eaten red meats in the world and enjoys great popularity in many developing countries, especially in Asia, Africa and the Far East (Talpur et al., 2008). India ranked second in goat meat production. Worldwide, the demand for meat and other animal products is increasing at a substantial

Fig 1: Different cuts of muscles of goats

rate driven by a combination of population growth, urbanisation and rising income.

To improve the public image of red meat and increase consumption the nutritive qualities have been promoted, namely that it is a good source of protein, iron and B vitamins. Improvements to the nutritional quality of lipids in ruminant meat have focused on the animal's diet in efforts to reduce the SFA content while enhancing the monounsaturated fatty acid (MUFA) and PUFA content with an n-6/n-3 PUFA ratio favourable for human health. Howe et al. (2006) have emphasized the importance of meat as a source of long-chain polyunsaturated fatty acids; (LC-PUFA, ≥ C20), particularly for populations with traditionally low intake of food from marine sources. Additionally, conjugated linoleic acid (CLA), a lipid natural to ruminant products, has been shown to have many bio-functional properties (Park, 2009). So there is a need of nutritional management for enhancement of CLA and PUFA acid in meat.

Strategies to improve fatty acid composition of meat

Any change in the process of lipolysis or biohydrogenation will influence the supply of their intermediate and end products, including CLA to the small intestine and ultimately their contents in the milk and meat. Dietary factors have been shown to be a prominent contributing factor in effecting CLA production in ruminants. The fatty acid composition of meat, milk and dairy products is highly dependent on the animals' diet (Khanal and Olson, 2004; Rana et al., 2012).

Effect on lipolysis and biohydrogenation

Replacement of forages with grain in the diet reduced the rates of lipolysis and biohydrogenation. Increased proportion of nitrogen in the diet resulted in increased rates of lipolysis and biohydrogenation by rumen contents in vitro. Lipolysis and hydrogenation reactions were more rapid with feed particles ranging from 1-2 mm size than from 0.1 to 0.4 mm size (Gerson et al., 1988). Biohydrogenation of fatty acids averaged 47 percent in the rumen of cows fed diets containing calcium salts of palm oil and 71 percent with diets containing fat from animal vegetable sources (Wu and Palmquist, 1991). CLA are highly correlated with either LA or α-linolenic acid. It is well known that LA and LNA are both precursors of the CLA isomer cis-9, trans-11 but the highest concentration of this CLA isomer generally obtained with LA rich diets. Factors affecting ruminal fermentation and microbial population are undoubtedly the keys to control the regulation of biohydrogenation and CLA synthesis (Hossain et al., 2017).

Effect of substrate on CLA concentration

Qiu et al. (2004) observed that with more LA available for CLA production, there will be higher CLA flow from rumen. Fellinar et al., (1995) found that overall rate of biohydrogenation of LA in vitro was 14.35 percent per hour, but declined to 1.2 percent per hour for each percent unit increase in LA added. So it is evident that high LA concentration in diet reduces biohydrogenation and increase post ruminal flow of CLA.

Pasture and conserved forages

Grazing animal on pasture, feeding fresh forages or increasing the amount of forage in the diet will elevate the percentage of CLA as a proportion of total fatty acid in meat from ruminants (Tyagi et al., 2008). CLA content was increased by 300 percent in milk when the green fodder in diet increased from 33 to 100 percent (Tyagi et al., 2007). When ruminants are raised on pasture and green fodder, their meat and milk contain much higher levels of n-3 PUFA and CLA as compared to products from animals raised in stall feeding with concentrates (Aurousseau et al., 2004). Fresh grasses provide C18 fatty acid substrate for ruminal biohydrogenation. Grazing beef steers on pasture or increasing the amount of silage in the diet increased the RA content in fat by 29 to 45 % as compared to control (Shantha et al., 1997). The increase in CLA content of beef varies with the quality and quantity of forage in the animal diet.

Feeding of oilseed

Inclusion of linoleic acid (C18:2) and linolenic acid (C18:3), which are main precursors of CLA in ruminant diet is one of the effective feeding strategies for increasing its production. Addition of oilseed to the diet has been proven to be an efficient method to increase the CLA content in muscle lipid. However, not all oilseeds exert the same effect. Kott et al. (2003) found that when lamb supplemented with safflower (6 percent oil from safflower seed) compared to control group the CLA content was significantly higher in longissimus lumborum muscle (4.1 vs. 9.0 mg/g of FAME).

Feeding of vegetable oil

Vegetable oils are equivalent to oilseed show similar effect to CLA. It has been reported that t-VA production in the rumen, as well as CLA content in milk and meat, was enhanced by feeding vegetable oils to cattle containing high levels of polyunsaturated fatty acids (PUFA), including soybean, corn, peanut, sunflower, linseed, mustard and fish oils (Duckett et al., 2002; Kathirvelan and Tyagi, 2009). A number of studies has shown that the inclusion of unsaturated vegetable oils at 4-5 % of ingested dry matter in

ruminants' diets brings a reduction of SFA and an increase of CLA and vaccenic acid (Mele, 2009). In beef cattle addition of 3 and 6 percent sunflower oil to a barley based finishing diet resulted increase CLA content in longissimus muscle (Mir et al., 2003). Vegetable oil influence CLA content in meat by supplying PUFA which are substrates for bacterial isomerisation and biohydrogenation. Bessa et al. (2007) obtained a significant increase for CLA concentration in lamb fat, after including soybean oil up to 7% of dry matter in the diets.

Feeding of fish oil

Feeding fish oil increase the n-3 long chain PUFA concentration in the intramuscular fat due to the high Eicosapentaenoic Acid (EPA) and Docosahexaenoic Acid (DHA) content in fish oil. Ruminal biohydrogenation of EPA and DHA is limited and therefore considerable amounts of this fatty acid are available for incorporation in to adipose tissue (Raes et al., 2004).

Effect of direct administration of Butyrivibrio fibrisolvens

Direct administration of high CLA producing Butyrivibrio fibrisolvens strain (109 CFU/head) isolated from Indian buffalo into crossbred kids able to increase CLA content of rumen fluid, longissimus dorsi, semitendinosus muscle and adipose tissue by 60.6, 30, 19.1 and 30%, total PUFA content by 38.4, 29, 28 and 11.6%, and reduce SFA content by 6.65, 10.5, 12.1 and 4.3%, respectively as depicted in Table 1 (Hossain et al., 2013).

Effect of plant extracts

Several plants extracts such as Cuminium cyminum, Terminalia chebula (Rana et al., 2011; Vasta et al., 2010; Miri et al., 2013) prevent the final step of biohydrogenation, so as to accumulate tVA. Thus a multipronged strategy involving high CLA producing strain with these extract will further enhance the possibility of producing ruminant meat product with high CLA content.

Health recommendations

Recommendations for optimal lipid intake to minimize metabolic related disease in humans are strongly related to lifestyle and constantly being reevaluated through new research findings. General guidelines have recommended that total fat intake does not exceed 30% of the daily energy intake. Recommendations for the different FA classes have suggested that SFA contribute less than 10% daily energy intake and that the PUFA/SFA ratio is higher than 0.4 (Smit et al., 2009). Substituting MUFA for SFA is encouraged; however, the intake of trans-MUFA should not exceed 3 g/day (Weggemans et al., 2004), or the equivalent of 1% of the daily energy intake (Willett and Mozaffarian, 2008). The total PUFA intake is recommended to be 18 g/day or about 6 to 7% of the daily energy intake (Chapkin, 2008). The recommended intake of PUFA is more complicated as the composition of the PUFA has been shown to play an important role in determining the associated health benefits. Simopoulos (2002) advocates a return to a pre-industrial n-6/n-3 PUFA ratio of 4:1 for optimal health benefits, compared to the current estimated 15:1 n-6/n-3 PUFA ratio typical of a Western diet. The dramatic change in the human n-6/n-3 ratio over time has been largely attributed to lifestyle changes. Wijendran and Hayes (2004) emphasized that the n-6/n-3 PUFA ratio was not as important as the absolute intake of PUFA to modulate metabolic risk factors. An intake of 450 mg/day of n-3 LC-PUFA has been recommended to meet the needs of an adult human (Givens and Gibbs, 2006). Suggested optimal PUFA intakes expressed as a percent of daily energy are as follows: 6% C18:2n-6, 0.75% C18:3n-3 with 0.25 to 0.5% of combined C20:5n-3 and C22:6n-3.

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BYPASS PROTEIN TECHNOLOGY FOR IMPROVEMENT OF LIVESTOCK PRODUCTIVITY IN RUMINANTS

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Introduction

India has limited feed resources including protein meals. The problem of feed shortage could be overcome to some extent by generating newer feeds, modifying the feeds and feeding conditions, and by manipulating the digestive tract especially rumen through active as well passive manipulations. While the active rumen manipulations are aimed at enhancing rumen microbial fermentation, especially fiber degradation by providing requisite nutrient metabolic environment for proliferation of cellulolytic microorganism in rumen. The passive rumen manipulations are aimed at reducing ruminal degradation of proteins, fats and starch by allowing these nutrients to escape rumen as such, and then get digested and absorbed from the lower tract. This forms the basis for bypass nutrient technologies. The aim of these technologies is to increase the efficiency of protein and energy utilization in dairy animals, in order to fully exploit their potential for growth and milk production. Measurement of DCP is considered inadequate and unsatisfactory means of assessing the protein value of the diet as no distinction is made between the digestion in fore stomach and in the small intestine.

Development of RDP and UDP system

There has been a conceptual change in protein metabolism, requirement and protein quality evaluation for ruminants during the last three decades. The subject was exhaustively reviewed by Chalupa, 1974 and Clark, 1974 who brought home certain truths about N metabolism in ruminants, the ones which had not received the attention of the scientist before.

Requirement of RDP and UDP for Ruminants

It has been realized that ruminal microbes do not supply adequate protein for satisfying the nutrient requirements of ruminants for their production, so for efficient utilization of protein feed should be formulated in such a way that it is able to provide 60 % UDP of the total protein in the diet (NRC, 1989). There are many factors that can influence the bypass protein content in forage. These are:-

Maturity

Numerous research projects have demonstrated that immature forage legumes and grasses contain more degradable and less undegradable protein than mature forages. Immature forages contain more non-protein nitrogen primarily composed of ammonia, nitrate, amines, amides, and free amino acids which are rapidly degradable in the rumen. With advancing maturity, true plant protein synthesis advances and the cell wall matrix becomes more complex, rendering forage protein less accessible to rumen bacteria and less degradable. These factors ultimately reduce degradation potential of forage proteins (Sampath, 1990).

Species

Species is also known to affect bypass protein content of forages. In general, legume protein is more degradable than grass protein. This is due in part to grasses containing more neutral detergent fiber which reduces rates of nutrient digestion (Malik et al., 1981). Within grasses, bromegrass and quackgrass appear to have greater levels of bypass protein, while perennial ryegrass protein appears to be quite degradable (Sampath, 1990).

Fertilization

Grasses assimilate soil nitrate (NO3) and ammonium (NH4) into non-protein nitrogen and true protein fractions. Increasing soil N supply increases forage N (crude protein). The increase in forage N (crude protein) is, however, disproportionate with the non-protein nitrogen pool increased to a greater extent than the true protein pool. Because non-protein nitrogen is readily degradable in the rumen, nitrogen fertilization generally reduces the amount of bypass protein. Fertilizing grasses with 0 or 120 lbs/acre of N resulted in increasing crude protein 1.5% units and increasing protein degradability (Garg et al., 2002).

Ensiling (Proteolysis)

When forages are ensiled, bacteria ferment the forage and breaks forage protein down into smaller fractions which are more degradable by rumen bacteria. This process is called proteolysis. Some researchers estimated that only 9% of forage macro protein molecules remain after fermentation. The effect of proteolysis can have a dramatic effect on the bypass protein content of forages. The concept of proteolysis can be demonstrated from a recent study from our laboratory . Alfalfa silage was made at three different maturities and wilted for 0, 10, 24, 32, 48, and 54 hours before ensiling. Ruminal degradability of ensiled forages was compared to a non-ensiled forage (NE).

Characteristics of bypass protein

The following characteristics are desirable for Bypass protein supplements for dairy herds,

- Rich in crude protein content.
- Optimal essential amino acid profile.
- Contain about 70-80% protein in a rumen undegradable form.
- Approximately 80%of the rumen undegradable protein in digestable form in the small intestine.

Methods of Estimation of Undegradeble protein

The undegradable or bypass fraction can be estimated in a number of ways which include

- laboratory enzyme procedure,
- use of cannulated animals where the amount of feed protein escaping digestion in the rumen can be measured,
- a nylon bag procedure where samples of the test feed are placed in porous bags which are then incubated in the rumen.

The use of proteolytic enzymes to estimate undegradable protein is attractive as the procedure can be carried out in the laboratory without the need for animals. A proteolytic enzyme is added to the test feed under controlled conditions in the laboratory. The rate and extent of protein digestion by the enzyme can then be used to estimate the degradable and undegradable protein content of the feedstuff. However, incubation time, enzyme level, sample grinding size, etc. can all influence the results obtained. Thus, results obtained using this in vitro procedure may not correspond with values obtained when the protein source is fed to the animal.

Animals fitted with a duodenal cannula can also be used to measure bypass protein in a feedstuff. The advantage of this procedure is that various types of proteins can be measured which are passing out from rumen. By using various markers bacterial protein and ammonia nitrogen can be measured. Total protein less bacterial protein and ammonia nitrogen is usually assumed to represent feed protein which has escaped digested in the rumen (bypass protein). Disadvantages of this method include cost associated with cannulated animals and the fact that the estimates obtained are for the total diet rather than the individual ingredients.

The bags are then washed, dried and the amount of protein remaining undigested in the bag is weighed. The proportion of bypass protein and degradable protein is calculated based on the amount of protein remaining in the bag after various times of rumen incubation. The advantage of this technique is that it is relatively rapid and it can be used to estimate protein degradability of individual feedstuffs as well as the total diet. However, degradability of protein supplements can be influenced by washing procedure, fineness of grinding, bag porosity, basal diet, substrate-to-bag surface ratio and contamination of bag residues by bacterial nitrogen and average (DIP) proteins through plant and animals sources through nylon bag technique.

Techniques to protect the protein from rumen degradation

On the basis of protein degradability data (in rumen) obtained by several groups of worker on large number of feed stuffs in India and other countries, it has been found that only a few feeds are good sources of bypass protein (having lover protein degradability), viz., maize gluten meal, cotton seed cake, fish meal, coconut cake and maize grain. Feeds like linseed cake, de-oiled rice bran, soybean meal and Lucaenea leaf mean (Subabool) are of medium protein degradability, while mustard cake, rape seed cake and groundnut cake are highly degradable cakes in rumen (Walli et al., 1999). While the proteins of lower protein degradability do not need any protection in rumen but highly degradable cakes need protection against attack of ruminal proteolytic enzymes, in order to improve their protein utilization by ruminants. There are several methods for preparation of bypass protein these are:

- Tannic acid treatment
- Browning of protein.
- Heat treatment of protein
- Formaldehyde treatment

Tannic acid treatment : when protein are treated with 2-4 per cent tannic acid it forms a insoluble protein complex which reduces enzymatic action by cellulytic bacteria in the rumen (Walli et al., 1999). But this method is not too much applicable.

Browning technique : The mixture of protein and xylose or other sugar are mixed (1:1 ratio) and heated at about 80-100 degree centigrade for 6 to 24 hours to reduce the rumen degradation and UDP increase from 29 to 70 per cent (Chatterjee and Walli, 2003) . Different protein require different degree of treatments (heat or chemical) for achieving appropriate level of UDP in the system .

Heat treatment : When protein feeds are heated, EAA groups within and between forms cross linkage which not only reduce the solubility but also reduce the surface of protein for bacterial enzymatic action. The heat treatment thought has the limitation of not being cost effective and may overprotect the proteins (Walli et al., 1999) tried to fine-tune the heat treatment of GN cake and soybean cake and found 1500 C for 2 h to be the optimum temperature – time combination. In the case of soybean, roasting of the seed can be popularized, provided some good model of roaster, which is capable of operating within the fine-tuned time- temperature combination, is developed. The

time–temperature combination of 1600C for 30 minutes seen to be effective for protecting soybean protein (Kumar et al., 2006).

Formaldehyde treatment : The aldehyde react with EAA groups and nitrogen terminal group to form Schiff bases and cross linkage between protein chains thus reduce the solubility of protein and protect it from bacterial enzymatic degradation. Once the treated protein enters into the high acidic medium in the abomasum the reaction is reversed and protein is degraded. (Chatterjee and Walli, 2003) also optimized the level of formaldehyde as 1.2 g/100 g CP for the treatment of mustard and groundnut cake, at which level the protein degradability is substantially reduced without having any adverse effect on post ruminal digestibility of cake protein (Kumar et al., 2006). The treatment enhance desired level of protein protection, bioavailability of essential amino acids, control salmonella and reduced mould growth and less expensive than heating.

Effect of Bypass protein on Nutrient Utilization

Effect on Amino acid availability

Research has shown that for higher producing cows, a desirable level of %UIP/CP can vary from 35- 40%. At ANC we strive for levels of 36-39%. It is important to understand that UIP is dependent on amino acid analysis of diet feedstuffs. For example, it is possible to have the proper %UIP, but if the ration is short a limiting amino acid, production benefits will not be realized. Conversely, if all necessary amino acids are present but not in a bypass form where they can get to the small intestine the added benefits will not be seen.

Effect on Nutrient intake, digestibility and Nitrogen Balance

Table : Nutrient intake, digestibility and n balance of lactating crossbred cows fed diets varying in rdp/udp ratio

Attributes	Treatments		
	T1 (71:29)	T2 (58:42)	T2 (44:56)
Nutrient Intake			
DM intake (kg/100kg B.W.)	2.46 ± 0.51	2.79 ± 0.17	2.67 ± 0.22
DM intake (g/kg $W^{0.75}$)	115.38 ± 18.52	123.87 ± 4.70	121.58 ± 6.93
CP intake (g/kg/ $W^{0.75}$)	12.90 ± 2.07	13.73 ± 1.43	13.93 ± 1.76
TDN intake (g/kg $w^{0.75}$)	74.93 ± 11.32	77.522 ± 2.94	75.85 ± 6.58
Nutrient Digestibility			
DM	66.49 ± 1.46	63.84 ± 3.41	64.22 ± 3.80
OM	70.28 + 1.32	67.41 + 3.19	67.87 + 3.51
CP	65.76 ± 2.24	64.22 ± 2.21	63.36 ± 2.62
EE	61.20 ± 3.28	58.33 ± 5.15	56.41 ± 4.43
CF**	59.41^{b} ± 1.39	54.95^{a} ± 1.65	55.42^{a} ± 2.98
NFE	76.85 ± 1.14	75.34 ± 2.79	76.16 ± 2.82
Nitrogen Balance			
N. intake (g/d)	213.78 ± 23.62	192.34 ± 25.32	210.12 ± 17.53
N outgo thorough faeces as % of N intake	34.39 ± 2.24	35.98. ± 2.22	36.78 ± 2.62
N outgo through urine as % of N intake	32.82^{b} ± 2.62	26.79^{a} ± 2.66	25.37^{a} ± 2.69
N retained as % of N intake	10.32 ± 6.88	11.87 ± 6.38	13.20. ± 6.01
N retained as % of N absorbed	15.73 ± 10.03	18.55 ± 9.42	20.54 ± 8.73
N outgo through milk as % of N intake	22.47 ± 6.42	25.36 ± 3.07	24.65. ± 2.96

(Chaturvedi and Walli ,2003)

Effect of UDP in milk yield and composition

Table: Daily average milk yield, fat and protein percent of feeding one kg bypass protein supplement

Parameters	Control	Experiment (UDP 74% of CP)
Average milk yield (kg)	14.1. ± 0.26	15.2 ± 0.28*
Average fat (%)	4.4 ± 0.10	4.6. ± 0.14**
Average Protein (%)	3.2 ± 0.00	3.5 ± 0.01*

(Garg *et al.*, 2002)

*(*P <0.05 , **P <0.01)*

On growth performance

Feeding of formaldehyde treated oil meals generally increases the growth rate of animals and the increase is often significant. A significant increase in growth rate was observed on feeding formaldehyde treated GN cake and mustard cake to goat kids (Mudgal and Sengar, 1980; Gupta and Walli, 1987) to crossbred calves (Kumar and Walli, 1994) and formaldehyde treated fish meal to buffalo calves (Tiwari and Yadav, 1994; Chatterjee and Walli, 2003). The growth rate could be as high as 25-30% than the carcass weight.

On reproductive performance

Because of the higher growth rate caused through bypass protein feeding, the young stock can attain early maturity to start the reproductive life at an earlier age. Apart from that, studies have shown that bypass protein feeding can improve the reproductive efficiency of breeding buffalo bulls and cross bred bucks, both, with respect to sexual behavior including libido score as well as the seminal attributes like ejaculate volume, mass activity and sperm count. Similarly, positive results were obtained in female goats, where the days to first postpartum estrus, days open and the number of services per conception decreased after feeding of bypass protein, This positive trend in reproductive efficiencies could be attributed to lower plasma ammonia concentration and higher amino acids availability observed in animals fed bypass protein, the former preventing adverse effect on conceptions rate in females, thought uterine pH and later, enhancing spermatogenesis in males (Kumar and Walli, 1994).

Economics of feeding bypass protein

The economics of milk production on feeding protected proteins was calculated by Garg et al., 2002 is as fallows

Economic of feeding bypass protein :

Average value of 14.1 kg milk in control with 4.4 % fat (@ 8.00/kg)	Rs. 112.77
Average value of 15.2 kg milk in experimental group with 4.6 % (fat @ 8.14/kg)	Rs. 123.88
Increase in gross income@ animal/day	Rs. 11.11
Additional feed cost	Rs. 1.50
Increase in daily income	Rs. 9.61

(Garg *et al.*, 2002)

It was observed that on feeding 1 kg protected sunflower meal net average daily income is Rs 9.61 per/animal/day.

Constraints in application of the Bypass protein

The addition of an undegradable protein source may increase the undegradability of the ration to the point where there is insufficient degradable protein available to the rumen microbes. Microbial protein synthesis decreases and there is no net gain in the flow of protein (amino acids) to the small intestine. This is not likely to occur, given the high degradability of typical Manitoba feedstuffs.

- The information regarding degradabilities of protein in all the raw material used in cattle feed are not get available and may be quite variable depending on Source, Manufacturing conditions, Presence of other compounds

- Easy laboratory test for protein degradability are not still available and still some considerable disagreement as to which method the best indication of the content of bypass protein in a protein level.
- There are insufficient data from feeding trials available on milk production per unit imput of bypass protein under the system commonly used by small farmers.
- Many protein meals are undegradable in the rumen,however their degradibilities in the intestine may be very low. This applies particularly to protein meal with high tannin content- such protein source are not good source of protein to the animals since much of the protein is lost in faeces.
- For the most efficient utilization of bypass protein for production , the essential amino acid to total N ratio must be high.
- The response of bypass protein depend on the digestible energy content of diet.. At low digestible energy feeding of high level of bypass protein will result in amino acid degradation for energy supply.

Conclusion

For the above discussion it is evident that CP and DCP measures or inadequate for preparing the ration to the ruminants specially, high yielder's. Concept of UDP not only provide increased amount of amino acid from the same level of protein in the diet without effecting the DMI , digestibility of nutrients and nitrogen balance, it significantly improved milk yield, fat and protein in lactating animals , growth in heifers and improved reproductive efficiency . Beside these UDP also help in economical productive performances of animal's in spite of some minor constrains while using UDP.

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ORNAMENTAL BIRD REARING : ALTERNATIVE SOURCE OF INCOME GENERATION

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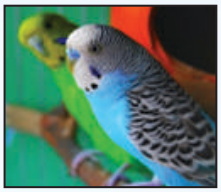
Introduction

Now a day the agro-ecological situation is characterized by complex diverse risk prone nature resulting in low production from monocropped agricultural land. In that case an alternative income path can save the farmers' from loss. Animal husbandry is one of the ways in which a farmer can earn extra income. But most livestock needs green grass, which is not available most of the time, and the price of whole grains is very high. In this context, the cultivation of Ornamental Birds is very profitable and the cost of food is very low.

Now a day the cultivation of ornamental birds like love birds, budgerigar, cockatiel, java, finch, etc. has become very popular in homestead (cage system) condition. Among them, the cultivation of budgerigar and finch birds is a safe and reliable way.

Monogamous means they spend their whole life with the same partner. Companions attract each other by feeding and tidying up. The upper part of their lips is red and it covers the lower part. They are usually 4 – 6 inches long and weigh 20 – 25 grams. Naturally the female lays one egg every two days. Usually the female birds sit down over the eggs and the male birds bring food for the female birds. The male bird eats himself and vomits the half-digested food in the bird's mouth. They need a lot of care after the baby is born. They remain blind after birth. In this condition the mother feeds them and keeps the baby warm. After 10 days, their eyes open. After about 3 weeks, the real hair on the bird comes.



Budgerigar

Love Birds

Finch

Cockatiel

Nature of Ornamental Birds : The following written characters of birds are seen -

1. **Stretching –** They occasionally spread their wings. This improves their blood circulation.
2. **Beak Grinding –** They often make noises on their lips while sleeping.
3. **Preening –** To keep themselves tidy, the birds apply oil substances from the "Preen Gland" at the base of the tail all over the body, making it shiny and good.
4. **Fluffing –** They rub their lips inside the soft hairs while tidying themselves before going to bed or before intercourse.
5. **Hearing & Stredding –** The beaks of these birds are always growing, so they keep rubbing their lips on other things so that they do not grow too much.
6. **Napping –** They usually sleep once a day for 15 to 45 minutes.
7. **Yawning –** Many times they make gape before or after sleep, it is normal for them.

Housing System

1. For 100 numbers of birds, a house of 15 feet long, 4 feet wide and 6 feet high is required.
2. If there is no place to keep a separate bird house, then those who have domestic chickens in their house, birds can be kept on the second floor of the chicken house. Bird house can also be made attached to the living room. It also provides care and benefits.

3. You have to put straw on the roof of the house.
4. Darma ceiling should be given under the tent.
5. The mold of the tent of the house should be extended up to 3 feet so that rain water does not enter it.
6. The boundary wall should be up to 1 feet height from the floor.
7. Wire mesh should be provided from 1 feet above the tent. It will be better ventilation.
8. The floor must be paved. When there is a soil floor, the birds start digging the soil floor.
9. The outside of the house should be surrounded by gunny 1 feet away. It will be used to prevent northern air.
10. Some stick should be hung in the house so that the birds can sit.
11. To clean the house, 300 grams of lime should be spread 1 week before.
12. Phenyl water should be sprayed in the house every 15 days.
13. When keeping birds, if there are insects in the house, 3 ml Butox should be mixed with 2 litter water and sprinkled.

Clay Clown to Keep Birds

1. You have to pay 1 clown for each pair of birds.
2. A hole about 4 inches in diameter should be placed at the top of the belly of the clown.

3. The distance between the two clowns should be 1.5 to 2 feet.

Feeder and Waterer

1. The feeder or feed container should be heavy so that it does not overturn.
2. You have to pay 4 containers for every 100 birds; 500 grams of food should be given in each container.
3. For every 100 birds 2 waterer should be given which contains minimum 200 ml of water each.

Feed (For every 100 birds)

1. Every day 1 kg of grain food should be divided into two containers.
2. 50 grams of soaked raw gram should be given.
3. 200 grams of Hincha or Kalmi or Thankuni vegetables should be given per week.
4. Adequate amount of Basil Leaves can be given daily.
5. One day in a week, 150 grams of soaked wheat should be boiled for 15-20 minutes before given.
6. One day in a week, 50 grams of Rice should be given and necessary medicines should be mixed with the Rice.
7. Spread 100 grams of Rock Salt on the floor every week.
8. Red Sand should be given in a container, which will help them in digestion.
9. One pot will contain 100-200 grams of lime.
10. They eat more food when there are chicks in the bird clown. So at this time you have to give more grain food.

Disease and Treatment

1. Lime Stool – (any one of the following drugs)

I. Meriquin Liquid – 5 ml once a day mixed with rice for 100 birds for 3 days.

II. Terramycin Capsule (500 mg) – 1 capsule once a day mixed with rice for 100 birds for 3 days.

2. Boils under the skin – A lot of the time around the eyes, under the jaw, there is a pus-filled wound on the face, in which case the best way is to remove the pus.
3. Feather Cyst – it is usually seen on the wings, but can also be seen on other parts of the body. It can be caused by genes or by hair follicle injury. It can happen again and again if not eliminated properly.
4. Egg retention and egg peritonitis – If the egg gets stuck, the inside of the egg should be removed with a narrow needle. In that case only the shell will be stuck, in that case if you put calcium in the mouth it will come out in 2 – 3 days. A many cases, the eggs are stuck and the oviduct comes out and the bird dies after drying. A little hot toast is good.
5. Bumble Foot – Many times the soles of the feet become thin and red, this is what happens in bacterial infections. This disease is cured by giving antibiotics.
6. Scaly face and legs – This painful situation is caused by the attack of parasites. They make these fibers and then small holes in the lips. This

disease is cured by giving Ivermectin.

7. Worm Infestation – Worms can cause various diseases to the birds, but it is not possible to give worm medicine in time.

Things to Know

1. Birds should be nurtured as female and male pairs.
2. When buying birds, you have to buy a healthy and strong baby less than 3 months old.
3. To identify males and females, at the age of 3 months, a pink tinge is seen on the lips near the head for females and a blue tinge is seen on the male birds.
4. Lays eggs from 3 months to 2 years of age.
5. Birds should not be kept in pairs after 2 years.
6. The bird lays eggs 3 times a year.
7. Birds can lay 6 – 8 eggs every time.
8. The baby hatches from the egg in 21 days.
9. If the child is 21 days old, it can be sold.
10. Large birds should be given large grains and small birds should be given small grains.
11. The blood pressure of birds is very high, so if any of their veins and sub-veins is cut, there is a lot of bleeding and it is dangerous. As a result, the blood must be stopped immediately.
12. Injuries can often cause air to build up on the skin beneath the air sac near the chest. In that case, the sir should be vented through a clean needle.
13. To avoid getting cold, 2 tips of Tetracycline Powder for 100 birds should be given mixed with rice 2 days a week.
14. Regularly mix calcium and vitamin together and give 5 ml for every 100 birds.
15. 5 grams of Electrolyte Powder should be given 3 days a week for 100 birds.
16. Liver tonic 3 ml per 100 birds should be given every 15 days interval of 3 days.
17. Albendazole Liquid (Dewormer) should be given 1 drop per bird every 2 months interval from the age of 2 months.

Conclusion

With the green revolution, now, Agricultural scenario of India as well as West Bengal have achieved to their highest potentiality. Climatic aberrations are also paving its bad effects on the farmer's production and productivity. In these circumstances, alternative livelihood generation in an adoptable, profitable, suitable and sustainable manner through animal husbandry is really praiseworthy and became a hope to the farming fraternity. Appropriate scientific aptitude along with proper marketing may prove this farming to be a future attraction for the upgraded and updated farming community.

EFFECTIVE PARASITE CONTROL – A FUTURISTIC APPROACH

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Introduction

Poultry and Livestock sector play an enormous role in the Indian national economy and in the socio-economic development of the country. According to 20th livestock census, total livestock population in India is 535.78 million (2019). It is increasing day by day due to its importance as an alternative source of income as well as food. The contribution of livestock in total GDP is 4.11%, Livestock in agricultural GDP is 25.6 % and that of agriculture in total GDP is 17.76 % (Basic Animal Husbandry Statistics, 2019-20). The country has made tremendous progress in terms of animal production in the field of dairying ,sheep/goat and poultry production. Recent survey has clearly indicated that the livestock growth at national level is increasing under animal husbandry sector compared to agriculture. There is a further scope of enhancement through improved nutrition, scientific management and strategic management of animal diseases.

Major parasitic infestation

Now-a-days shift in modern farming towards increased productivity and intensification in the livestock industry including cross breeding, importation of livestock/ germplasm has favored the spread of diseases. The major constraint in livestock sector is animal health problem; animal diseases are causing huge economic losses to the farmers especially in small ruminants in the tropics and subtropics. In India an average >300 helminth spp. parasitize livestock, mostly with multiple infections due to conducive environment for their development (Singh and Srivastava, 1977). Among all the different helminths, gastro-intestinal nematodes (GIN) are the major parasites drawn attention. The GIN impact animal health and production by number of ways viz, anorexia or reduced voluntary feed intake, mortalities in lambs/kids, digestive and absorptive disturbances, reduced productive and reproductive performances, anaemia, pathological changes in GI tract, loss of body weight and immunological disturbances. All these factors impact on the livelihood of farmers by economic losses. Environmental conditions in tropical/sub-tropical countries like India are conducive for rapid development and long time survival of the infective stages of parasites throughout the year and the animals are continually exposed to heavy infections.

Conventional treatment approach and its limitations

Classically, the problem is tackled by chemical intervention. However, due to tremendous biotic potential and short regeneration time, the animals get reinvested within no time. Thus repeated medication is required to keep up the animal health by using chemical dewormers/anti-helminthtics. This will enhance the development of resistance to all available drugs.. Moreover, drug residues in food products of animal origin affect Public and environment health. On the other hand, there is increased demand for animal origin food, which are free from chemical contaminants. Presently, economic animal production further gets a jolt due to absence of very few effective vaccines against parasites. All these factors forced the researchers to think over some alternative methods of management of parasites, which will be eco-friendly and sustainable. Considerable research has been conducted on various aspects of alternative methods, which include exploitation of genetic resistance, nutritional management, biological control of helminths etc. Sound knowledge of the epidemiology of the disease, effective and early diagnosis followed by judicious use of appropriate parasite control method can only form an effective basis for the control of parasites.

Alternative approach of parasite control

Following information need to be gathered before developing a strategy for the control of parasitic infection: Nature of infection (whether sporadic, endemic or ubiquitous). Type of life cycle of the parasite i.e., direct or indirect. Previous history of the infection in the flock. Geo and agro-climatic data (particularly rainfall, temperature and humidity) of the area. Managemental practices being followed at the farm. Nutritional level of animals of the flock.

a) Copper oxide wire particle

Alternative methods for control of nematodes Control of a parasite means to minimize the intensity or to prevent further spread of an existing infection in animals and decreasing number of infective larvae by adopting suitable parasite control measures. Presently, control programme is targeted by three main principles to “break” continuity in cycle of nematodes 1. To eliminate the worms in the host using non-conventional anthelmantics viz, feeding copper oxide

wire particles, natural from of anthelmintics from plants, nematophagous fungi and diatomaceous earth 2. To improve the host resistance and/or host resilience by immune-nutrition, genetic selection and available vaccines against parasites 3. To reduce contacts between the host and the parasite infective stages (L3) can be achieved by diluting the parasitic risk, exploiting the biological rates of development or death of free living stage and increasing larval death rate. Copper oxide wire particles (COWP) Initially COWP were developed to treat copper deficiency in animals but the potential interest to limit parasite infections was noticed in treated animals. It act indirectly on adult nematodes through the increased copper status in the host, or directly by potentially penetrate the cuticle of *Haemonchus contortus* due to increased copper in the abomasums of host. Few researchers reported 75-96% reduction in adult *H. contortus*, 56% reduction in *Teladorsagia circumcincta* in lambs and goats after administration of COWP. Even though, COWP showing promising results against few nematodes, copper toxicity is the limiting factor for the use of COWP in sheep. So, should be used accordingly.

b) Plant secondary metabolite

The naturally available plants, rich in anthelmintic properties are usually exploited by farmers. Few anti-parasitic effects in plants contribute to slowdown the dynamics of infections and also cause positive consequences for host resilience due to presence of one or more plant secondary metabolites (PSM). The PSM such as condensed tannins, cysteine proteinases are thought to be important elements. The cysteine proteinases are present in plants such as papaya, pineapple and figs and documented effect in several host species against a variety of nematodes which, causes damage to the cuticle of nematodes. The condensed tannins are secondary metabolites, acts directly by reduction in the development, viability, motility, and migratory ability of parasite larvae. However, indirectly they increase protein availability in the lower gastrointestinal tract through the protection of dietary protein from rumen degradation.

c) Integrated approach

The sustainable integrated approach is a systemic application of two or more technologies in environmentally compatible and cost effective manner to control parasite population. It aims to control multiple parasite to achieve maximize quality, quantity and sustainability of production and long term profitability. It includes... strategic and tactical use of anthelmintics careful management of grazing lands Anthelmintics remain an indispensable component of worm management programs, but they must be used in combination with alternative approaches. Usually deworming time, doses are most important. The time of deworming depending on the age of animal, parasite load in animals and managemental practices being followed.

d) FAMACHA system

Alternative treatment approaches to nematode parasite control is the FAMACHA system. Hereby, the lower eyelid of the sheep is examined and treatment administered only if signs of anemia are present. FAMACHA© is used to determine anthelmintic treatment in animals, depending on colour eye chart was developed in South Africa. It was developed as a tool for anthelmintics resistance and integrated parasite management against the barber pole worm. It was mainly developed for sheep, but should work with goats with slight modifications. The tool is a simple chart that allows individuals to determine the degree of anemia by comparing the colour of an animal's ocular mucous membranes to colours of eyes on a chart. The level of anemia is a key sign of the degree of parasitic infection with *H. contortus* . The FAMACHA© technique reduces the number of treatments because only animals showing physical signs of infection are dewormed. It identifies worm susceptible animals for culling and slows anthelmintics resistance, as worms have less exposure to the drugs. Only those sheep found to have pale mucous membranes, and where subsequent determinations showed haematocrit of 15% or below, were drenched to prevent death. This system not only saves anthelmintic usage, but animals could also be identified for culling. Such an approach dramatically reduces the selection for anthelmintic resistant parasite populations, as nearly three quarters of the flock remained untreated, with the result that their worm populations were not exposed to drug selection. The FAMACHA system can be used to control *H. contortus* throughout its endemic region where worm control is troubled owing to prevalence of anthelmintic resistance.

e) Grazing management

To reduce the environmental contamination by parasites, pasture management like rotational grazing, burning the grazed pasture is widely practiced parasite control strategies. Since these methods provides opportunity for dual livestock species parasite control, such as with sheep/cattle interchange grazing. A pasture grazed by cattle and/ or horses is also considered safe, since sheep/ goats and cattle/ horses do not share the same parasites (Barger, 1999). The alternative grazing of new born & resistant animals of same species also reduces nematodes population on pasture as young animals are most susceptible to parasitic infection. In rotational grazing, pastures are sub-divided and the animals are intensively grazed for

short period but, generally does not help to control internal parasites unless pasture rest periods are long enough (>70 days). Similarly, in controlled grazing permit pastures to rest and soil life to function well, so that contamination can be reduced. Access of clean un-grazed pasture first to young animals such as lamb, calf or kid may reduce the risk of parasite infection.

Apart from these control strategies, Segregation of the clinical case and its proper treatment by using anthelmintic drug (dewormer). All apparently healthy animals should be given preventive medication. Vaccinate the animals against the parasites for which the vaccines are commercially available. eg. Lung worm vaccines (Dictol, Difil), RakshaVac-T, TickGARD etc.

f) Biological control

Biological control of parasites can also be tried by use of predacious fungi, breeding of ducks and fish in ponds infested with the snails etc. Breeding of genetically resistant breeds of animals should be tried. Adopt suitable preventive measures to prevent further spread of infection. Immunological control One of the major objective and dream of the researches is to control the parasitic diseases or reduces the loss in productivity by manipulating the host immune response.

g) Immunological control

Immunological control is an ideal methods to control any kind of infection/s. But due to many inherent nature of the parasite and lake of proper knowledge on parasite immunity, the progress was very slow and many a times outcome was very poor. Initially, some anti-parasite vaccines, such as lung worm vaccine, Miller's vaccine for dog hook worm and anti-coccidial vaccines, were developed which is based on whole parasite, live attenuated type, having many disadvantages like very less self-life, problem of contamination, maintenance of cold chain, aesthetic problem, and reversion of its virulence and most importantly generation of parasite stage for vaccine preparation.

h) Recombinant vaccine

To overcome this problem, recombinant vaccine against parasite was first successfully developed against Taenia ovis, but there is no market and finally called as 'orphan vaccine'. Later, vaccine against *Rhipicephalus (Boophilus) microplus* was developed in Australia as TickGARD and Cuba as Gavac, the first recombinant vaccine against arthropod. Recently, subunit vaccine against *Haemonchus contortus* was developed by using native H11 antigen, an integrated parasite gut membrane glycoprotein, in Australia. The vaccine called 'BarberVax' was commercially manufactured by the Albany parasitology laboratories, Australia since 2014. In future, we may see progresses in this field where young researchers are well equipped with the knowledge of bioinformatics, molecular biology, computational biology and host-parasite relationship.

Conclusion

The development of effective, sustainable control options for parasite has thus far proven difficult, may be due to a lack of fundamental knowledge on parasite biology and the host-parasite relationship and partly due to high degree of biological plasticity of the parasites themselves However, sustainable control of parasite may be achieved by an improved under-standing of the biology of the parasite and long-term impact of parasite management strategies where each of various methods can be combined in an integrated format. The following strategies can be applied to prolong the life of the parasiticides, which is a major component in IPM at present. Indiscriminate use of anthelminthics develops resistance against the particular drug, so care has to be taken while using them. Reduce the use of anthelminthics by adopting other control measures viz, managemental control, nutritional control, immuno-logical control, genetic control etc. More than one method of control should be used in the form of integrated parasite management to prolong the shelf life of anthelmintic and to achieve proper parasite control. Since, IPM is more efficient successful control measure against gastro-intestinal nematodes.

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EVALUATION OF DIFFERENT METHODS FOR VIRAL DNA EXTRACTION FROM SHEEP POXVIRUS INFECTED CELL CULTURE

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Abstract

Several methods have been reported for viral deoxyribonucleic acid (DNA) isolation but limited information is available on quick and simple isolation of sheep pox virus (SPPV) genomic DNA. The primary aim of this study was to evaluate and compare four different DNA extraction methods using phenol/ chloroform/ isoamyl alcohol, lysis buffer, proteinase-k as conventional protocol and commercial nucleic acid extraction kit for isolation of SPPV genomic DNA from infected cell line. Viral DNA was extracted from clarified culture supernatant of SPPV infected lamb testis (LT)/Vero cells, harvested after exhibiting distinctcytopathic effect (CPE), using earlier mentioned methods. The quantity and quality of extracted DNA by each protocol were assessed which shows maximum yield of DNA was obtained in proteinase-k method. In this study, proteinase-k protocol was found to be comparatively effective method in terms of yield of SPPV DNA which is free from Polymerase Chain Reaction (PCR) inhibitors.

Key words : Sheep pox virus, DNA isolation, phenol/chloroform/isoamyl alcohol, lysis buffer, proteinase-k.

Introduction

An efficient genomic DNA extraction method that yields pure and high-quality DNA is required for the success of PCR, cloning, sequencing etc. and subsequent diagnosis of viral diseases (Mc-Orist et al., 2002; Koontz et al., 2015; Koontz et al., 2019).Comparison of manual and automated nucleic acid isolation methods for poxviral DNA (Espy et al., 1995; Armson et al., 2017) and for other viruses (Yagmur et al., 2015) also have been reported but limited information is available on sensitive and rapid isolation of SPPV genomic DNA therefore. SPPV with genome size approximately of 150 kb, causes serious disease in domesticated sheep, notifiable to The World Organization for Animal Health. The present study was targeted to compare four different DNA purification methods for genomic DNA extraction from SPPV infected cell culture.

Materials and Methods

The SPPV virus strain

Three isolates of SPPV-strain (SPPV-Jaipur, SPPV-Ranipet and SPPV-RF) were obtained from the repository of the Division of Biological Product, Indian Veterinary Research Institute, Izatnagar, Bareilly, UP. All the three strain were propagated in lamb testis (LT) primary cell culture while SPPV-Jaipur and SPPV-RF strain was also adapted and propagated in Vero cell line. The infected cells were harvested 4th-5thdays post infection when 80% CPE was observed. For DNA extraction, infected cells were subjected to two cycles of freezing and thawing for effective cell disruption and cell free virus lysate was recovered (Chifney et al., 1973) by low speed centrifugation at 1820g for 10 min at room temperature. The cell debris was discarded and one ml of SPPV infected cell culture supernatant from all the five samples was taken further for viral DNA isolation.

Extraction of viral DNA from infected SPPV virus culture

Among these four different protocols, 1st and 2nd method were phenol/chloroform/isoamyl alcohol extraction procedure and cell lysis protocol followed as per Bergallo et al. (2006) report. Third method involves addition of 10µl of 10% Sodium Dodecyl Sulphate (SDS) to one ml of viral harvest with final concentration of 0.1%. Proteinase-k was added at final concentration of 20µg/ml and incubated overnight at 37ºC or in water bath at 37°C for 3h. Enzyme was inactivated by heating the sample at 65ºC for 20 min. An equal volume of buffer saturated phenol was added and shaken vigorously for about 3 min. It was centrifuged at 14240gfor 5 minutes to separate layers; the upper aqueous layer was transferred to a new tube and to it equal volume of chloroform was added and shaken vigorously. Same procedure of centrifugation at 14240gfor 5 minutes, is done and the upper aqueous layer was aspirated in a new tube and 1/10th volume of 3M sodium acetate was added to it. Twice the volume of 100% ethanol was added and shaken gently for few seconds before keeping at -20° C for 30 min. The sample was centrifuged at 14240gfor 5 min. to get the pellet which was washed with 500µl of 70% ethanol for 3 min. at 14240g. The pellet was air dried and further re-suspended in 80µl nuclease free water. The last method, DNA of SPPV was isolated following the protocol mentioned in user's manual of the commercial kit. Care was taken to isolate DNA from 1ml of the infected SPPV culture and eluted in 80µl of the elution buffer.

Evaluation of DNA quantity and quality by polymerase chain reaction (PCR)

After the isolation of DNA by four different protocols, DNA quality and quantity of all five samples were measured by nano-drop spectrophotometer and was also checked in 0.8% agarose gel. The isolated SPPV DNA were further taking as template and the RNA polymerase subunit19 gene (RPO19) was PCR amplified using specific primer.PCR was conducted with 25µl reaction volume containing 2.5µl of 10X PCR reaction buffer, 0.5U/µlTaq DNA Polymerase (GeNet Bio, Korea), 20mM Magnesium Chloride (GeNet Bio, Korea), 10mM of each dNTP (dATP, dCTP, dGTP and dTTP); 0.5µl of each forward and reversed primer (10pMeach) and 2.5µl of template DNA. Sufficient amount of nuclease free water (Sigma, USA) was added to make the final volume of25µl. The cycling conditions were as follow: 95°C for 5 min, 30cycles of 94°C for 30 sec, 49°C for 30 sec, 72°C for 30 sec followed by 72°C for 7 min in a thermal cycler.

Primer	Sequences	Length	Predicted Amplicon size
Forward primer:	GACGAAGATGCGAGTGATGA	20 bases	410bp
Reverse primer:	TCTGTTACGGATAACAATTCTCCA	24bases	

Table 1 : Details of primers used for PCR reaction

Result and discussion

Nucleic acid analysis through PCR is very essential method in research and diagnostic field (Lantz et al., 2000). The reliability of DNA amplification depends on unbiased extraction procedures so that DNA should be free from any PCR inhibitors like micro-organism, biological or chemical substances, and contaminating materials (Wiedbrauk et al., 1995).The quantity (Barbas et. al., 2007) and quality of extracted DNA ,isolated from all SPPV isolates propagated in LT (lamb testis)/Vero cell culture i.e. SPPV-Jaipur (LT), SPPV-RF (LT), SPPV-Ranipet (LT), Jaipur (Vero) and RF (Vero), were assessed by nano drop spectrophotometer (Table: 2) and 0.8% agarose gel containing 0.5 µg/mL ethidium bromide (Fig. 1). Out of these four methods the Proteinase-k method which was based on enzymatic digestion of protein, was found to yield good quantity of DNA approximately 3-5 times more than the other methods (Table. 2). The commercial kit yielded good quality DNA but the yield was less as compared to all the four protocols. The other two methods viz. phenol /chloroform /isoamyl alcohol extraction (≈56.8ng/µl) and use of cell lysis buffer (≈47.5ng/µl) yielded around three times less DNA than Proteinase-k method and also the 260/280nm absorbance ratio was ≈1.64 and ≈1.38 respectively indicating low purity of DNA contaminated with either traces of phenol or protein. The DNA extracted by proteinase-k method from all samples of SPPV gave good PCR amplification (Fig. 2) indicating absence of PCR inhibitors. Similar work was done by Bergallo et al., (2006) for polyoma BK virus and he reported phenol/chloroform/isoamyl alcohol as most effective method.

Samples	Methods							
	Protocol:1		Protocol:2		Protocol:3		Protocol:4	
	ng/µl	$A_{260/280}$	ng/µl	$A_{260/280}$	ng/µl	$A_{260/280}$	ng/µl	$A_{260/280}$
Jaipur(LT)	54.0	1.62	25.5	1.33	75.7	1.89	32.9	1.81
RF(LT)	43.7	1.58	46.0	1.43	67.4	1.75	28.5	1.75
Ranipet(LT)	28.4	1.65	28.6	1.38	53.3	1.77	12.1	1.76
Jaipur(vero)	90.5	1.69	85.9	1.47	542.0	1.95	78.4	1.84
RF(vero)	67.3	1.67	51.7	1.29	201.7	1.79	62.8	1.83
Average	56.78	1.64	47.54	1.38	188.02	1.83	42.94	1.80

Table 2: Quantity and quality estimation of viral DNA isolated by various methods

To check whether the isolated DNA is free from PCR inhibitors or not, a gene fragment of SPPV i.e. RNA polymerase subunit 19 (RPO19) of product size 410 bp was amplified at standardized cyclic conditions. As a result, the gene fragment of 410 bpsize of all the samples were amplified and showed good intense band in 2% agarose gel (Fig.2).

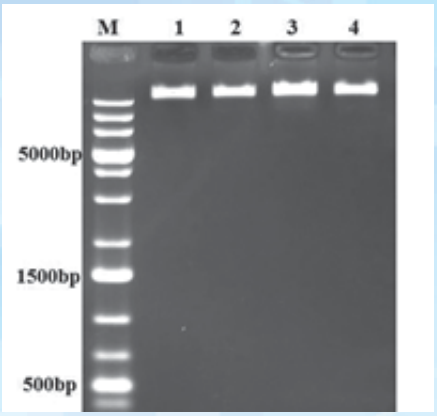

Fig.1: SPPV virus DNA isolated using four different protocols on 0.8% agarose gel where Lane M is for 1 kb plus DNA ladder and Lane 1,2,3& 4 are showing intact bands of viral DNA isolated using Protocol 1st,2nd,3rdand 4threspectively.

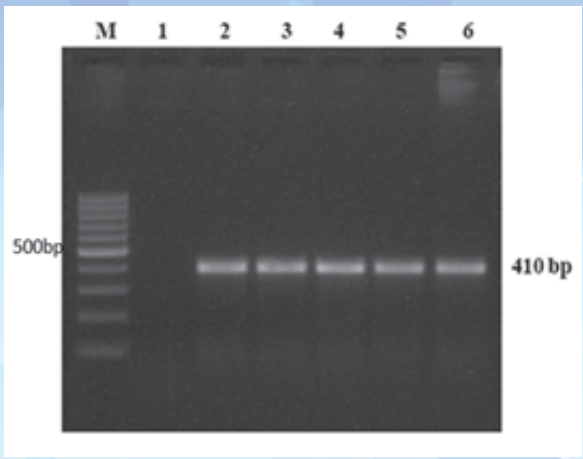

Fig.2: Agarose gel (2%) showing amplification of RNA polymerase subunit 19 (RPO19) gene. Lane M: 100 bp DNA ladder; Lane 1: Negative control; Lane 2 & 3: Jaipur (LT and vero); Lane 4 & 5: RF (LT and vero); Lane 6: Ranipet (LT)

CONCLUSION

In the present study, our results demonstrated that Proteinase-k extraction procedure is the most efficient in terms of yield and purity of viral DNA than other method used as a routine tool.

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PSEUDOMALARIA IN DOMESTIC PIGEONS : A CASE REPORT

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Introduction

Haemoproteus is the largest genus of avian haemosporidian parasites distributed all over the world (Krizanauskiene et al., 2013). It is the most common intracellular haemo parasite of birds, reptiles and amphibians being reported from various bird species (Burry-Caines and Bennett, 1992). Among the birds, pigeons are considered to be attractive and raised as pets for thousands of years as a means of religious and/or cultural symbols. Pigeons suffer from variety of metazoan and protozoan infections. In this regard, *Haemoproteus columbae*, transmitted by *Pseudolynchia canariensis*, a hippoboscid blood sucking fly affects domestic as well as wild pigeons. The disease caused by *H. columbae* in pigeon is called as pigeon malaria or pseudomalaria and is lethal chiefly in young pigeons. The parasite widely occurs in pigeons of tropical and subtropical regions. The disease can be diagnosed by observation of halter or crescent shaped gamonts in the erythrocytes partially encircling the nucleus of the host cell (Soulsby, 1982). From Indian perspectives, a higher prevalence of *H. columbae* in pigeons has been documented form Uttar Pradesh and Mumbai (Shinde et al., 2008; Jahan et al., 2011). However, informations regarding occurrence of pseudomalaria particularly, in pigeons is scanty from this geographical part (West Bengal) of the country. This paper highlights the diagnosis of severe *H. columbae* infections in adult domestic pigeons by Giemsa staining method.

Materials and methods

Case history and sample collections

Domestic pigeons of village Nischintapur & Rajpur Sonarpur Municipality under Sonarpur block of West Bengal were brought to the Block Animal Health Centre, Sonarpur with a history of reduced feed intake and symptoms of dullness, dyspnea, torticollis and diarrhoea. Blood samples were collected aseptically from wing vein by vein-puncture in vials containing EDTA. The body hair coat was also carefully searched for the presence of ectoparasites, if any. Whole blood in EDTA and Blood smear samples were sent to ADDI, South 24 Parganas by the VO, BAHC, Sonarpur for diagnosis of the disease.

Staining of blood smears

Thin blood smears were prepared and subjected to Giemsa staining method following the standard protocol (Soulsby, 1982). Briefly, the blood smears were prepared, air dried, fixed in methanol, stained with Giemsa and examined under oil immersion lens of compound binocular microscope (100×) for the detection of haemoprotozoan parasite.

Results and discussions

Infections with *Haemoproteus* sp. are generally asymptomatic owing to low pathogenicity of the parasite. Clinical examination of the pigeon revealed dullness, depression, dyspnoea, torticollis and frequent diarrhea as reported by other workers (Nematollahi et al,. 2012; Varshney et al., 2014). Careful examination of body hair coat revealed the presence of louse fly underneath the wing which acts as the vector for *H. columbae*. Microscopic examination of Giemsa stained thin blood smears revealed intra-erythrocytic halter shaped gametocytes encircling the host cell nuclei (Fig. 1). The present observations are similar with that of earlier workers (Weisman, 2007; Nematollahi et al., 2012; Borkataki et al., 2013).From India, a higher prevalence of *H. columbae* in pigeons has been documented form Uttar Pradesh (U.P.) and Mumbai (Shinde et al., 2008; Jahan et al., 2011). Jahan et al. (2011) reported 55.63 % infectivity of *H. columbae* in rock pigeons of Bareilly regions, U.P. The mean parasitic load of the infected pigeons was 1.78 gametocytes/ 100 RBC's. Nandi and Bennett (1997) also documented a prevalence of 54.4% *H. columbae* infection in pigeons of U.P. Similar result (58.33 %) has been stated by Shinde et al. (2008) from urban localities of Mumbai, India. Surveys indicate that ecological factor, vector abundance and susceptibility of the bird might play a role in determining the incidence and levels of parasitaemia in different regions.

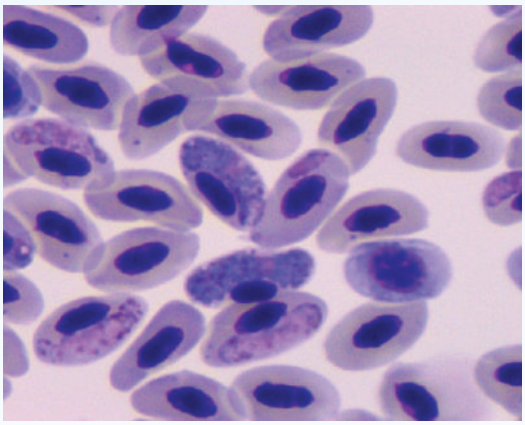

Fig. 1: Blood smears showing *Haemoproteus columbae* gametocytes in the RBC

Treatment

Affected pigeons were successfully treated with Chloroquine @ 15 mg /kg body weight daily indrinking water for two days with multivitamin supplementation. 2% permethrin was used as dip to remove vector fly from the body of the pigeons. After one week following treatment, the affected pigeons recovered with normal feed intake and absence of torticollis and other symptoms.

Acknowledgement

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INCIDENCE OF CANINE BABESIOSIS AND EHRLICHIOSIS IN AND AROUND CHINSURA, HOOGLY, WEST BENGAL

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Abstract : Two hundred and eight exotic dogs were screened for haemoprotozoan diseases with canine babesiosis and canine ehrlichiosis from January 2020 to December 2020, twenty were found clinically positive for either babesiosis or erlichiosis indicating an overall incidence of 9.61% for the diseases. The characteristic clinical symptoms observed were pyrexia, anorexia and anaemia. Laboratory examination report revealed anaemia, neutrophilia . On microscopic examination Babesia as *B. canis* and Ehrlichia as *E. canis* were detected in Giemsa stain of peripheral blood smear as well as ticks were collected from the inner aspects of the ear for specific identification and collected ticks are identified as *Rhipicephalus sanguineus* from there morphological characters. In the present therapy we used of subcutaneous injection of imidocarb @ 0.5 ml/10 kg body wt (repeated after 14 days), oral administration of doxycyclin tablet @ 10 mg/kg body weight for one month along with supportive therapy using hematinic syrup, multivitamins and liver extract for one month. All clinically positive dogs were negative to either babesiosis or ehrlichiosis after 28 days.

Introduction

Canine babesiosis is one of the important life threatening tick borne haemoprotozoan diseases of dogs. The disease is caused by intraerythrocytic protozoa under the genus *Babesia*. *Babesia canis* and *Babesia gibsoni* are two most common species known to infect dogs which are transmitted by *Rhipicephalus sanguineus* tick. B. canis is more common in United states whereas B. gibsoni has been found to infect dogs in Middle East, Africa, Asia and Europe (Harrus et. al ,2012). A wide variation of clinical sign has been reported in canine babesiosis including anorexia, lathergy, anaemia, icterus, vomiting as well as loss of body weight. Clinicopatholical abnormalities are ascitis, haemoglobinurea, hypoglycemia, azotemia and disturbances of acid base balance (Irwin, 2010 , Reddy et. al ,2016). Canine Ehrlichiosis or canine rickettciosis or tropical canine pancytopenia is an another important tick borne rickettcial disease caused by intracellular organism under the genus *Ehrlichia*. *Ehrlichia canis* and *E. ewingii* are important species under the genus *Ehrlichia* (Das and Konar, 2013).

Very little information is available in treatment of these parasitic diseases where complete elimination of these parasites is possible. In most cases, even after proper diagnosis and treatment, dogs become chronic carriers or present with recurrent episodes of acute infection. In this case report, we present a complete therapeutic guideline after diagnosis through clinical symptoms, laboratory analysis and microscopical studies of canine babesiosis and ehrlichiosis. Finally we observed that after 28 days of given therapy dogs are negative to babesiosis and ehrlichiosis.

Case history clinical observation

The present study was carried on twenty clinical cases of babesiosis and ehrlichiosis in exotic dogs mostly Labrador and German Shefard at the State Animal Health Centre, Chinsura, Hoogly from January 2020 to December 2020 . The animals were dull and depressed with common symptoms of pyrexia (ranges 104°F – 106.2°F) ,anorexia and anaemia. The animals were not responding to common antibiotics.

Treatment was done as follows

1. Inj. Imidocarb-1ml S/C; @ 0.5 ml/10 kg body wt. (repeat after 14 days)
2. Tab. Doxycyclin 200mg- ½ tab BD; @ 10 mg/kg body wt (for 4 weeks)
3. Syp. Hemobest-1 t.s.f. B.D. daily orally for 1 month
4. Syp. Zipvit-1 t.s.f. B.D. daily orally for 1 month
5. Syp. Livotas-1 t.s.f. B.D. daily orally for 1 month

Results and Discussion

Peripheral blood smear of dogs prepared from EDTA- anti coagulated blood and stained with Giemsa staining method and examined microscopilly and revealed presence of large form of babesia species B. canis and presence of small spherical cocco-bacillary ehelichian inclusion of circulatory monocytes which were identified as E. canis . The stained blood smear was also used for total and differential WBC count and hemoglobin was estimated in all 20 infected animal. The hematology finding revealed anemia (PCV percentage =26.29±12.28 and hemoglobin 8.1±3.2 in g/dl –Table-1and reference values according to the reports of Duncan , 1986 and Morgan,1992).

Table-1 : Hematology of 20 dogs infected with B.canis and E. canis co-infection

Hematology parameter	Result	Ref.values
PCV (%	26.29±12.28	37-55
Hemoglobin (g/dl)	8.1±3.2	12-18
WBC (/µl)	15628±533	6000-17000
Neutrophils (/µl)	14100±410	3000-11500
Lymphocytes (/µl)	3140±1240	1000-4800
Eosinophils (/ µl)	390±270	100-750
Monocytes (/ µl)	1276±380	150-350

In the present study, it was observed that the clinical signs against canine babesiosis and ehrlichiosis were similar to the published work performed by Damodaran,2006 as well as Das and Konar,2013. But in our case, coffee coloured urine or epitaxis were not found. However after 14 days of treatment with given drugs, animals are found positive to babesiosis while blood slides were examined under light microscope. Imidocarb was repeated after 14 days. After 28 days of treatment, all animal were negative to both babesiosis and ehrlichiosis in microscopical examination. In conclusion, imidocarb and doxycyclin are effective against coinfection of canine babesiosis and ehrlichiosis.

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A RETROSPECTIVE STUDY ON CUTANEOUS HEMANGIOSARCOMA IN 21 DOGS

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Abstract : Hemangiosarcoma is an aggressive neoplasm of vascular endothelium. The present study was conducted in 21 dogs in around Kolkata to explore clinicopathological characteristics of cutaneous Hemangiosarcomas and to evolve rational therapeutic management. This retrospective study demonstrated that cutaneous Hemangiosarcomas showed a predilection in lower abdominal region [Odd ratio: 34, P-Value : 0.013], Boxer where the breed showed greatest prevalence [Odd ration : 10.54, P-Value : 0.023]. There is a significant influence of doxorubicin adjuvant chemotherapy showed more favourable prognosis.

Key words: Cutaneous Hemangiosarcomas, adjuvant chemotherapy, doxorubicin

Introduction

Hemangiosarcoma (HSA) is a malignant neoplasm of vascular endothelial cells (Moulton, 1978). In dogs prevalence of this neoplasm is 0.3% to 2% (Waller and Rubarth, 1967; Pearson and Head, 1976). The spleen, liver and right atrium are primarily affected but cutaneous HSA are now so uncommon. It was reported that 14% of this HSA are cutaneous origin (Brown et al., 1985). There are few studies regarding clinicopathological behaviour and treatment of cutaneous form of the tumour (Hargis et al., 1992; Culbertson, 1982).

The purpose of this study to explore various gross, histopathological characteristics of cutaneous HSA and also to evolve rational therapeutic and prognostic management.

Materials and Method

Materials of this study where collected from various Veterinary Hospitals located within Kolkata for the period from January 2021 to January 2022. Overall 21 HSA were detected during this study period. The complete details of the cases such as breed, age and sex alongwith anatomical positions of the neoplasms were recorded for further analysis. Primary tumour mass were surgically removed and at the time of suture removal (10 to 14 days after surgery). 12 dogs were treated with doxorubicin (30mg/m2) diluted in 150 ml of 0.9% NaCl via slow intravenous infusion every 3 weeks for a total of 5 cycles. Butorphenol (0.4mg/kg i.v.) given 15 min before doxorubicin treatment to reduce vomition. Simalteniously, 9 dogs were treated with surgery alone. The histopathological evalution of HSA was obtained by excisional biopsy in all dogs.

Statistical analysis of breed, sex and tumour location predilections were performed using odd ratio analysis (Kleinbaum et al., 1991). Survival curves (obtained by Kaplan-Meier method) of 1 year were computed for further evaluating correlation between these two treatment group (Surgery alone and Surgery followed by adjuvant chemotherapy) (Kaplan and Meier, 1958). Comparisons of survival characteristics were made between these two subgroups by means of log-rank testing of discrete variables. For all statistical analysis, P-value of ≤ 0.05 was consider significant.

Result and Discussion

Mean age of the 21 dogs was 9 years and there were 11 male and 10 female. In the present study cutaneous HSA occurred on ventral abdomen (n=17), prepuce (n=2), pelvic limb (n=1), forelimb (n=1) and appear to be more common in Boxer (n=14), Labrador (n=3), Golden retriever (n=2) and German Shephard (n=2). Grossly tumours were varied from firm to soft fluctuant masses, redish-purple nodules or papules [fig 1]. The surface was often ulcerated and inflamed. Histopathologically the neoplasms were composed of blood field vacular sinusoids, surrounded by pleomorphic endothelial cells [fig 2].

Fig.1: Cutaneous Hemangiosarcoma with ulcerated suface

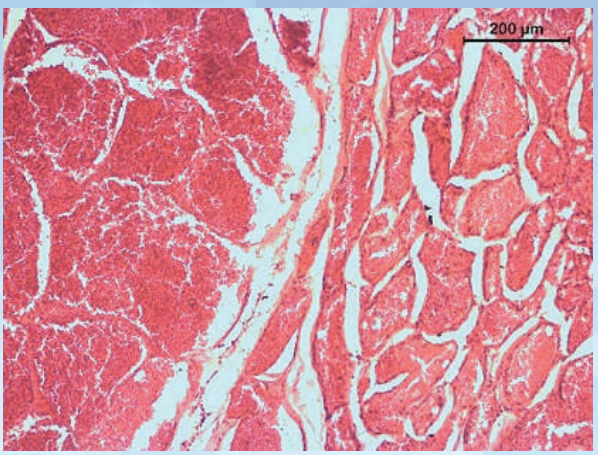

Fig 2: Blood filled irregular shaped vascular sinusoids in Cutaneous Hemangiosarcoma, H and E X 100.

In this study, based on odd ratio analysis, cutaneous hemangiosarcoma mostly occurred on the ventral abdominal region (odd ratio:34, P-value=0.013). Our results tends to agree with the report of 13 beagles in which lower abdominal region was mostly effected (Culbertson, 1982). Cutaneous tumours showed a predilection of lightly haired skin, when compared with hair skin (Hargis et al., 1992). Our study is also in agreement with this findings in which 18 of 21 (90%) of the tumours occurred in ventral abdominal or preputial skin. In the present study Boxers were the breed showed greatest prevalence (80%) and developed more cutaneous HSA than did other breed [odd ratio=10.50, P value:0.023]. Similar results has been reported by Ward et al., 1994. It was reported that dogs with sparsely haired skin developed more dermal HSA (Hargis et al., 1992).

In this study log-rank test, 8 dogs died in the group surgery alone and 2 dogs died in the group treated with doxorubicin, 3 were censored. The chi-squared statistics was 5.96 with associated P value 0.015 ($P \leq 0.05$). Therefore, grouping variable (i.e. Doxorubicin) has a significant influence on survival time. Survival was worse in the dogs having surgery alone. In this study addition of doxorubicin after surgery showed a survival benefits [fig.3].

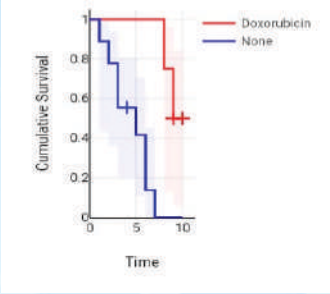

Fig. 3: Kaplan- Meier Survival analysis showing doxorubicin adjuvant therapy was more effective than surgery alone(P value= 0.015)

Conclusion

Our study suggest that in and around Kolkata dogs belongs to Boxer breed were much more predispose to cutaneous Hemangiosarcoma, greater percentage of tumours occurred in ventral abdominal region and dogs received doxorubicin adjuvant chemotherapy showed more favourable prognosis than surgery alone.

Acknowledgment

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EIMERIOSIS IN CATTLE & HAEMATO-BIOCHEMICAL CHANGES : A CASE REPORT

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Abstract : Bovine Eimeriosis generally causessudden severe mucus and/or blood containing diarrhoea in young calves but in occasionally it can occur in adult cattle (Constable et al., 2017). The present case was a two years old 258 kg Jersey crossbred cow complained with bloody diarrhoea and anorexia for two days. Laboratory examination of dung revealed presence of unsporulated oocysts of Eimeria spp. The haematological and biochemical parameters revealed anaemia (PCV 18%, RBC Count 3 x 106/µl and Hb 6 gm/dl),hypoproteinemia (5.2g/dl), hypocalcemia (7.9 mg/dl) and hypophosphatemia (2.3 mg/dl). The animal was treated with potentiated sulfonamide, Metronidazole,intravenous fluids and haematinicsfor four days which led to partial improvement of condition.

Key words : Eimeriosis, PCV, Bloody diarrhoea

Introduction

In dairy calves, eimeriosis occurs under overcrowded and dirty, wet conditions, through faeco-oral route. For most species of farm animals, the infection rate is high, and rate of clinical .disease is usually low (Constable et al., 2017). Many cattle are sub clinically infected, resulting in considerable economic losses (Joyner et al., 1966). Coccidiosis is uncommon in adult cattle, but occasional cases and even epidemics canoccur, sometimes in dairy cows that have calved 6 to 8 weeks earlier (Constable et al., 2017). Moist, temperate, or cool conditions favour sporulation, whereas high temperatures and dryness impede it (Constable et al., 2017).The prevalence of coccidiosis in cattle and buffaloes has been well reported from different parts of India (Nambiar and Devada, 2002; Singh and Agarwal, 2003). Eimeriosis in adult cattle was also previously reported from India (Reddy et al., 2013). But in adult cattle reports are lacking information about severity of the condition in terms of low PCV, RBC, Haemoglobin and other biochemical parameters. The present report is focused on the severity of blood loss and abnormality of biochemical parameters associated with *Eimeria* spp infection.

Case History and Observation

The case was presented with the history of sudden onset of bloody diarrhoea and anorexia for last two days (Fig-3). On clinical examination there was normal body temperature (102ºF), tachycardia (Heart rate-110/min), normal respiration rate 24 /min, pale mucus membrane, resilient rumen consistency, reduced rumen motility (1/3 min), rumen liquor pH 7 and reduced rumen protozoa (+ or 1-10/field in 10X; FIG-2) was observed. There was large watery, mucus containing, bloody faeces (Fig-1 & Fig-4) was present and persistent straining during defecation was present. The blood was collected from jugular vein and haemoglobin, PCV, RBC level was estimated by colorimetrically (Drabkin's Merthod), Wintrobe's tube method and Neuber's chamber respectively. The serum was separated and calcium, phosphorus and total protein were estimated by commertial kits following OCPC method (Gitelman, 1967), Modified Gomorri's method (Fiske and Subarrow, 1925) and Biuret method (Doumas, 1975) respectively. Five gram of faeces was collected from rectum and direct smear was prepared and observed under microscope for oocyst. The laboratory evaluation of samples gave clear picture of anaemia where RBC count was 3×106/µl (normal 5.1-7.6×106/µl, Constable et al., 2017), haemoglobin level was 6 g/dl (8.5-12.2 g/dl Constable et al., 2017) and PCV was 18% (normal 22-33% Constable et al., 2017). In biochemical parameters calcium, phosphorus and total protein levels were 7.9 mg/dl (normal 9.7-12.4 mg/dl Radostits et al., 2008), 2.3 mg/dl (normal 5.6-6.5 mg/dl, Radostits et al., 2006), 5.2 g/dl (normal 5.7-8.1, Constable et al., 2017) respectively. Microscopic examination of faecal smear revealed presence of unsporulated oocysts of *Eimeria* spp.

Treatment and discussion

On the basis of clinical examination and laboratory diagnosis the disease was diagnosed as eimeriosis. Eimeriosis in bovine causes bloody diarrhoea and straining during defaecation (Constable et al., 2017). In bovine eimeriosis the temperature may be normal or subnormal (Constable et al., 2017). The anaemic condition and hypocalcemia further explains the reason of tachycardia. The hypophosphatemia and hypocalcemia occurred due to reduced dry matter intake caused by anorexia which is explained before by Constable et al., 2017. Hypocalcemia is further substantiated by reduced rumen motility(Constable et al., 2017).The degree of hemorrhagic anemia in eimeriosis is variable, depending on the amount of blood lost (Constable et al., 2017). In experimentally

infected calves the haematocrit value is reported to as low as 12.9% (Bangoura and Daugschies, 2007). The value of RBC and haemoglobin level in blood as low as 3×106/µl and 6 g/dl respectively are reported in limited number of cases of Bovine eimeriosis earlier to this report. Hypoprotinemia is previously reported in sheep and goats with eimeriosis (Tafti and Hashemnia, 2017). Reduced rumen protozoa is may be due to anorexia. The case was treated with intravenous Potentiated Sulfonamide (Sulphomethoxazole and trimethoprim combination) according to Saravanan et al., 2016 which was reported to be effective against coccidiosis in buffalo calf. The case was also treated with intravenous metronidazole @ 10 mg/kg BID for controlling secondary anaerobic bacterial infection (Reddy et al., 2013). Intravenous fluid (inj. RL 2000 ml and inj NS 500 ml) was given @ 10 ml/kg (Reddy et al., 2013) to counteract fluid and electrolyte imbalances. Parenteral (intramuscular) haematinics inj. feritas (Iron sorbitol citric acid complex, folic acid, hydroxocobalamin acetate) @ 10 ml OD was given which was also previously incorporated in treatment of coccidiosis in adult cattle (Reddy et al., 2013). The treatment was followed for four days which led to gradual improvement of feed intake and bloody diarrhoea was controlled (Fig-5), after which the animal was taken back to owner's home. Prognosis of the anorectic animal with low PCV and bloody diarrhoea is guarded but our present case showed signs of improvement during therapy period.

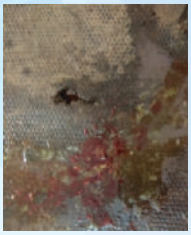

Fig-1

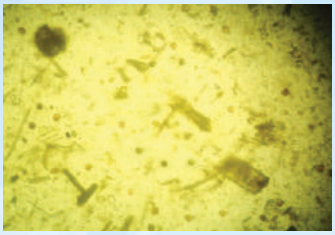

Fig-2

Fig-3

Fig-4

Fig-5

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A NATURAL ALTERNATIVE TO PRESENT-DAY ANTHELMINTIC DRUGS : CYSTEINE PROTEINASES WITH A SPECIAL REFERENCE TO GASTROINTESTINAL NEMATODES

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Introduction

Parasitic infections cause great morbidity and mortality, as well as economic, losses globally, especially in developing countries than infectious diseases both in man and animals. Even with the advancement of modern medical practice, the statistics are not falling due to lack of awareness of hygiene practice, sanitation, and proper healthcare; another reason is resistance to a small number of anthelminticsavailable to combat the vast number of helminths. about half of the world's population is infected by gastrointestinal nematodes. In humans, there are almost 3.5 billion cases worldwide, of which 450 million are seriously ill with a majority of children and 44 million are pregnant women with hookworm. About 125000 deaths occur per year mainly due to *Ancylostoma duodenal*, *Necator americans*, or *Ascaris lumbricoides* in contrast to 300-500 million malaria cases per year. Over the last fifty years, the world has witnessed 39 million disability-adjusted life years lost to GI nematodes compared to 34.1 million to measles or 35.7 million to malaria. Despite there being less public awareness about helminthic diseases than any other infectious or protozoal diseases because most of the time the infected individual remains asymptomatic and in spite of being debilitating the helminths produce chronic diseases which are not directly fatal.

Over the last 50 years, the number of cases has been increased with a majority in developing countries correlated with lack of hygiene, poor education, health care, and close coexistence of humans and animals. In addition, warm and humid climatesfavor the survival of free-living larval stages of GI nematodes. This type of climate is seen in tropical and subtropical countries, most of which are developing.

Consequence of GI nematode infection

A. Zoonoses

This nuisance is predominantly associated with rural areas of the developing world where animals live alongside human in conditions of overcrowding and poor hygiene, lack of medical and veterinary services, and unawareness of zoonotic diseases. One important zoonotic nematode infection is toxocariasis by a dog parasite *Toxocara canis* where the majority remains asymptomatic but some develop fatal diseases like visceral larva migrans, ocular larva migrans and covert toxocariasis where they cause migration of nematodal larva to visceral organs and causing tissue necrosis, chronic liver disease, edema, hemorrhage, and eosinophilia. Other nematodes are *Ancylostoma caninum* seen where people tend to walk barefooted etc.

B. Anaemia

The majority of anaemia cases are due to hookworms where people mostly rely on agricultural labour for the main family income. Heavy *Trichuris trichiura* infection has also been seen to cause iron deficiency anaemia whose severity is greater in the pregnant woman due to naturally increased iron requirement during pregnancy. Anaemia decreases the physical ability to carry out daily works of life, leading to poor nutrition and it can also cause death and abortion.

C. Malnutrition and economic loss in the livestock sector

Nematodes while feeding on the host damage the intestinal mucosal epithelial cells resulting in less nutrition absorption by the host leading to loss of appetite, stunted growth in both human and animal hosts. There is a huge loss of production from livestock too which directly affects the economy. A single dose of albendazole to helminth-infected children has improved their growth rate, weight, physical fitness, appetite, and activity (Stephenson et al.,1993). Following a regular deworming schedule in farms can greatly improve milk and meat production of animals and can also regularize the process of coming to heat.

Control of GI nematodes

Major chemical compounds to combat nematodes are termed antinematodal drugs, which can be further classified into the following groups as per their chemical structure and mode of action:

1.**Benzimidazole :** a) Benzimidazoles-Albendazole, fenbendazole, oxfendazole,mebendazole etc. (b) Pro-benzimidazoles- Febantel,netobimin, thiophanate.

2. **Macrocyclic lactones :** a) Avermectins-Ivermectin, doramectin, selamectin etc. (b) Milbemycins-Milbemycin oxime and moxidectin.

3. Imidazothiazoles- Tetramisole,levamisole etc.

4. Tetrahydropyrimidines- Pyrantel,morantel,oxantel.

5. Organophosphorus compounds- Crufomate, haloxon etc.

6. Piperazines- Piperazine and diethylcarbamazine
7. Arsenicals- Melarsomine, arsenamide etc.
8. Substituted phenols and salicylanilides - Disophenol, closantel etc.
9. Octadepsipeptides- Emodepside
10. Aminoacetonitrile- Monepantel
11. Miscellaneous drugs-Hygromycin B, phenothiazine, toluene, etc.

Problems with currently available anthelmintics to control nematodal infection

a) Development of resistance

Resistance to the all-major classes of anthelmintics used in livestock is widespread. Particularly in Africa, Australia, New Zealand, Asia, and South America; there is now a potential danger of resistance in helminths of humans. Resistance is a genetically determined decline in the efficacy of an anthelmintic against a population of parasites that is generally susceptible to the drug, and this is particularly worrisome because there are few new drugs at the clinical trial stage of development.

b) Social and economic factors

The expense of anthelmintics in relation to family budget is one of the major problems in developing countries. Though in some parts of this price is being subsidized by the governments. Even so, cheaper alternatives like tetrachloroethylene are being used by family members which areitself less efficient and are used in sub-optimaldoses thus encouraging the resistance.

Alternative of conservative anthelmintics

One alternative may be planted latices rich in cysteine proteinase which has been used by indigenous people in the tropics for control of GI nematodes both in man and animal. These lattices are found from species of fig, papaya, milkweed, pineapple. *Ficus glabrata* latex is a well-known anthelmintic in central and South America. *Ficus laurifolia* has also been used against *Trichuris*, *Taenia, Ascaris,* and *Enterobius*. Papaya (*Carica papaya*) and pineapple (*Ananas comosus*) has been used to treat chicken, dogs, pigs, humans with intestinal parasites

Some plant sources of cysteine proteinases

Plant	Location	Enzyme
Carica papaya	**Latex, unripe fruits**	**Papain, chymopapain, glycyl etc.**
Ananas comosus	**Fruit, stem**	**bromelain, Ananin, comosain.**

With the onset of resistance to the synthetic anthelmintics interest in this area of research has now been rekindled although satisfactory trials in humans that conform to contemporary standards are still lacking. In one rare example, in the Amazon basin, the crude latex of *F. glabrata* was administered orally for three consecutive days at 1mL/kg. After treatment there were reductions in egg output of *Ascaris* (85%), *Strongyloides* (72%), *Ancylostoma/Necator* (55%) and *Trichuris* (67%) almost matching the efficacy of the modern, synthetic anthelmintics used as a positive control. No adverse side effects associated with the use of fig latex were noted (Hanson et al., 1986). Studies in livestock are rare but anecdotal stories suggest the successful use, as early as the 19th century of the crude latex of *C. papaya* against ascarides, tapeworms, whipworms, and hookworms. In two well-documented trials, a single dose of crude latex of C. papaya provided comparable anthelmintic efficacy to the currently available synthetic anthelmintics, with respect to reductions in the egg output and worm burden in pigs infected with *Ascaris suum*.

In spite of the studies describing the efficacy of extract of papaya and fig, their mechanism of action is not yet assessed. This is important because current drug legislation requires an understanding of the mode of action of candidate drugs before registration.

Proof that the active principles in these extracts were cysteine proteinases was demonstrated by using purified cysteine proteinaseif cysteine as a reducing agent was present it showed anthelmintic activity (Stepek et al., 2005). In addition, the specific inactivator of the papain family of cysteine proteinases, L-trans-epoxysuccinyl-leucylamido-4-guanidino-butane completely inhibited the anthelmintic activity. It was also demonstrated that this mechanism was specific to cysteine proteinases as the anthelmintic property was not shown with aspartic or serine proteinases found in the mammalian alimentary canal.

Conclusions

In the present scenario control of GI, nematodes are inadequate due to many constraints. The inevitability of fewer new drugs will complicate the future control of GI nematodes. In this case, studies about alternative drugs like cysteine proteinases should be encouraged.

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BEHAVIOR MANAGEMENT IN DOG

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Introduction

`Most of the people buy or adopt puppy with lots of love and take care about their diet, health check up and protect their puppy from all that could harm, but very few of them conscious about the behavior of their puppy. We must keep in our mind that a badly behaved dog is an unwelcome nuisance. In today's fast paced life we all lead pressured lives and most of us are so self absorbed with our own problems that we fail to notice that our dogs are equally prone to get stressed. Like in people, stress is bad for dogs. Stressed animals are more likely to get sick and lives shorter lives.

All puppies go through a crucial phase of learning roughly between five week to four month age. At this stage the dog's mind is primed to learn to accept new experience. Dog that miss out on this crucial learning stage can often end up as fearful dogs that are afraid of new people, experience or even new places. Puppies that are raised in isolation, such as in garage, an isolated dog kennel or even in an empty room, have little exposure to human or other dog often end up with behavioral problems. Socialising a puppy involves teaching it how to behave in a socially acceptable manner with other people and animals. A good social skill has to be taught-a pup must learn to enjoy the company of the people, other dogs and being comfortable in different places and situation. Unfortunately this socialisation period coincides with the vaccination (inoculation) period and most of the veterinarian suggests barricading the puppy in the home and forbidding it from meeting strangers and other dogs. They mainly concentrate on proper immunization and prevent the pup to acquire a fatal disease. We must be very careful with our pups in this time regarding combination of socialization and timely vaccination. A good pet parent will ensure he does as much as possible to grow his puppy grows into a friendly and confident dog.

Socialising a puppy

There is no hard and fast rule to socializing a puppy. Pet parent have to keep in mind that they have to expose their pup as many different places, environment, people and animals but in a controlled manner under pleasant circumstances and with positive consequences. Each step should be taken slowly and if puppy getting nervous or unhappy, it is best to remove it from the situation rather than forcing it to face its fear. There are some general guides which may help the pet parent to grow their puppy into a happy and confident adult.

At Home

Socialization process begins that very day of arriving puppy at home. New experiences like ride in car/bike, meeting with new family members, trip to vet for routine checkup need to be handled carefully. Situations should be regulated at a controlled speed and don't expose it too much too fast. If the puppy getting stressed it will show some sign like excessive yawning, panting, lip licking. In that case allow your pup to get some rest in a quite spot. Over a period of weeks puppy may be exposed to object around the house specially, noisy home appliances like mixer, hair drier etc. Invite your friends, relatives and neighbor and get them to interact with the puppy in a positive manner. Always give a treat for obeying command.

Meeting people

Puppies that haven't been exposed in a positive manner to a wide variety of people-different ages, ethnic communities and colours can grow up being suspicious of particular people. Make sure your pup gets to meet and has pleasant experiences with as many different people as possible, for example, men in beards, women in flowing saris, children, screaming toddlers, a person in a wheelchair or walking with the aid of crutches, garrulous teens, etc. If you can take your pup to friend's homes and let your friends reward your pup with treats and praise for good behavior, it will learn that people are not to be feared and can be great fun to be around. It is important to get your puppy to interact with service people like postman, garbage collector, maid servant too.

Going places

In the first week of puppy it is necessary to take to as many different places as possible like children park, crowded street, a festive fair, a mall, a beach, football game as creative as you can. Let your pup take in the sights, sounds and smell in a new place and wait till he/she is relaxed and comfortable in that environment before rewarding him with treats, praise or even a game with toy. If he/she spooks at something, you may choose to wait in that place for a while or remove the puppy to a safer and quieter spot and reintroduce later depending on the level of fear he/she exhibits. Allow strangers to meet him/her so that he/she gets plenty of pleasant experience.

Meeting other dogs

Allow your puppy to interact with known vaccinated, well behaved adult dog with close supervision. Do not

allow your puppy to play rough, nip, irritate the older dogs. A sensible mature dog will have a great influence on yours pup's developing play behavior around other dog as well.

Stress factor for dogs

Dog tend to get stressed in situation where they feel out of control or unable to cope like excitement, pain, threat or anger can induce tension in a dog. A dog that lives in a house where there is constant fight, unhappiness or tension can pick up stress within minutes .A dog that is re-homed from one place to another in a short span of time, arrival of new baby in the family or loss of a family member can also cause a dog to worry.

Conclusion

A well socialized dog is fun to be around, welcome everywhere and is happy confident dog that will make the most of life. Investing a bit of time and effort into your puppy when it is young will reap rewards when the dog is older.

PRESENT STATUS OF FEMALE CATTLE AND MILK PRODUCTION IN WEST BENGAL

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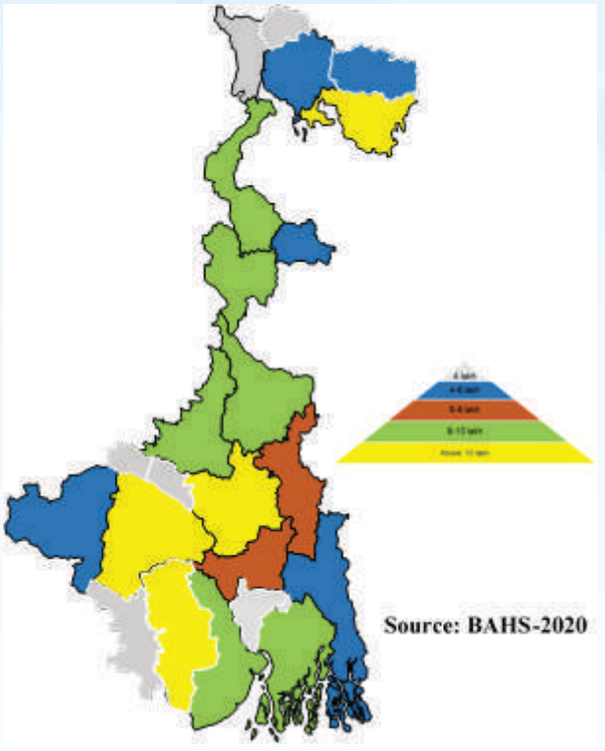
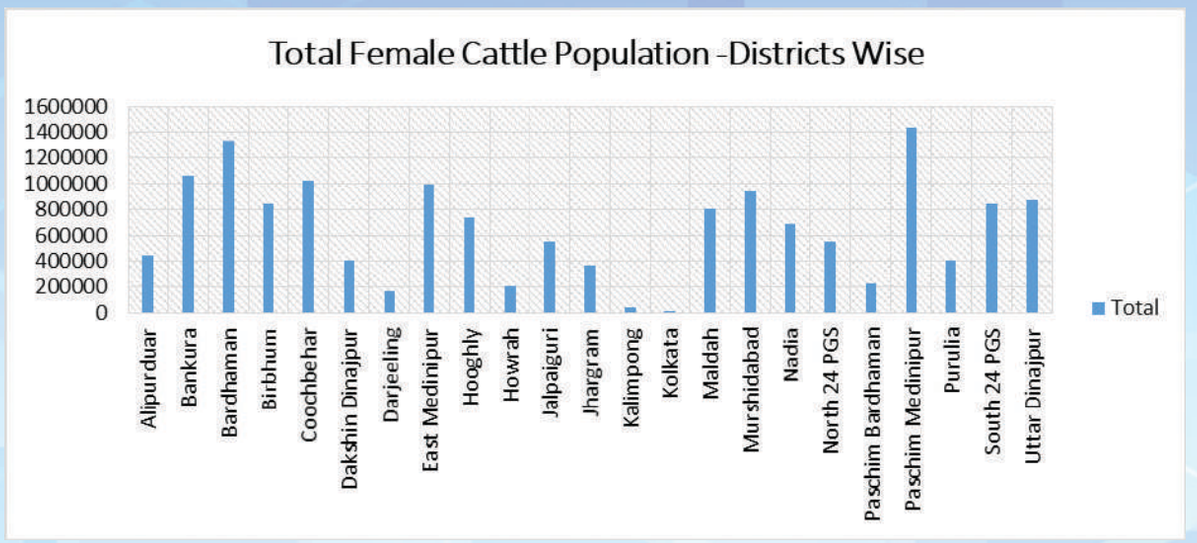
Introduction

West Bengal has strong animal husbandry practices among the rural population. Livestock plays a very supportive role in the rural economy. Dairy husbandry has key allied practice with main agricultural operations among the rural farming community. Milch cattle are reared to utilize chief agricultural by-products as feed resources as output cow milk is obtained. The paddy straw and cattle rearing have a strong correlation due to low input cost. Previously cow milk is used as liquid milk but in recent times value-added milk products gaining the marketplace. As a consequence, dairy farming creates livelihood opportunities among the farming community. Presently, several dairy cooperatives are formed and the private player came into the dairy business from collection to sale and marketing of milk and milk products that generate income and self-employment in rural youths. So, in this technical note, we will highlight the current status of dairy cattle and their production quality in the state of West Bengal.

Female Cattle Population

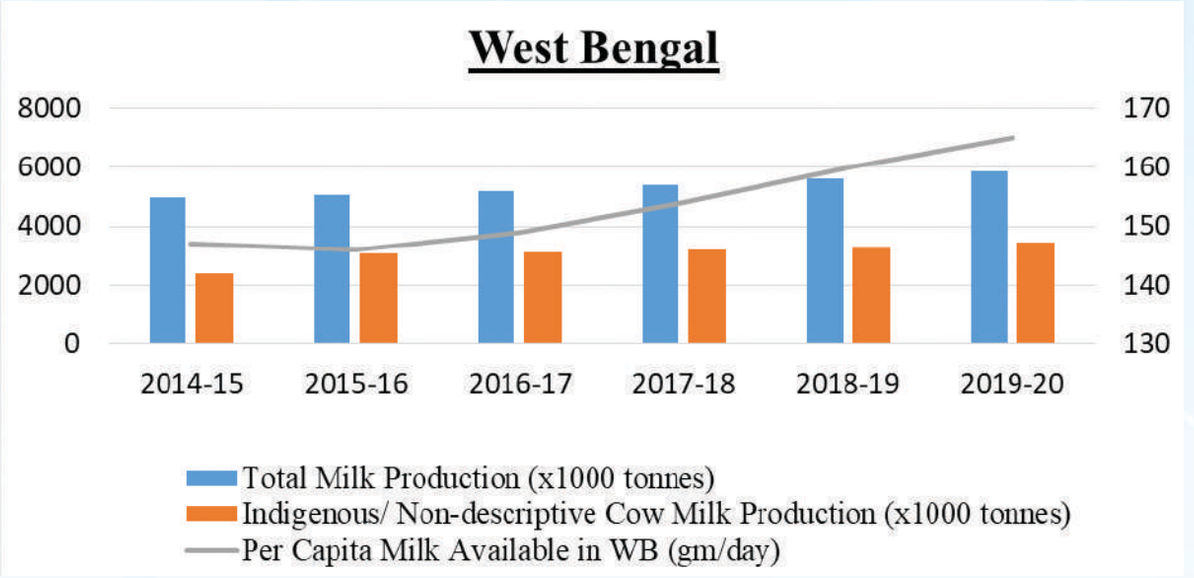
There are 15.52 % jumped in cattle population (19thvs 20th Livestock Census) in West Bengal. We stand at 1st position in Indiaas 19.1million cattle population.Total female cattle is 14.96 million in our state whereas PaschimMedinipur contributes the maximum number of female cattle of 1.43 million. Here, all districts are divided into five parts according to total female cattle population i.e. Yellow colour – above 10 lakhsdistricts (PaschimMedinipur, Bardhaman, Bankura and Cooch Behar); Green Colour – 8 to 10 lakhs districts (PurbaMedinipur, Murshidabad, Malda, Uttar Dinajpur, Birbhum and South 24 Parganas); Red Colour – 6 to 8 lakhs districts (Hooghly, Nadia); Blue Colour – 4 to 6 lakhs districts (North 24 Parganas, Alipurduar, DakshinDinajpur, Jalpaiguri, and Purulia); White Colour- below 4 lakhs districts (Howrah, Kolkata, Darjeeling, Kalimpong, Jhargram and Paschim Bardhaman).





Milk production

India is number one at milk production in the world. The per capita milk availability stands on average 406 gm/day for India but in state-wise comparison, in West Bengal per capita availability is 165 gm/day. Total milk production is 5868.61x1000 tonnes in West Bengal at 2019-20.



Conclusion

Total dairy animal increased that indicates there is huge potential to improve per animal production by upgrading our existing animals with suitable breeds and various government-sponsored schemes to become a milk resilience west Bengal.

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DIFFERENT TRATMENT SCHEDULED IN ANOVULATORY AND DELAYED OVILATORY INDIGENOUS COWS OF WEST BENGAL

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Anovulation and delayed ovulation are two important factors foe causation of repeat breeding syndrome in cows. These two disturbances are the the result of insufficient release of LH hormones by the pituitary gland. In west Bengal poor farmers cannot purchase and maintain costly crossbred cows due to their poor economy.it has been observed that good percentage of their cows suffer from repeat breeding due to ovulatory disturbances and remain infertile. This leads to economic loss of poor farmers.

Anovulation is a condition in which despite the occurrence of normal cyclic changes including manifestation of oestrus, the graffian follicle does not ovulate in spite of its normal size. In delayed ovulation the time of ovulation extends beyond normal 12 hours after the end of oestrus and may even be more than 48 hours. The consequence of ovulatory disturbances is either the oocyte is not liberated for fertilization or it is liberated too late so that fertilization becomes abnormal due to ageing of spermatozoa in the female genital tract.

It has been observed from the studies of different workers that the incidence of anovulation and delayed ovulation varies from 5.5 to 66 percent in cattle. Yet, the information in the respect of the method of diagnosis and treatment of these two ovulatory disturbances is very scanty. The most drawbacks for the diagnosis of these two conditions is that the affected animals need frequent gynecological examination of genetalia. This involves too much labour on the part of the breeders and clinicians.

It has been widely acknowledged that the levels of macro elements in the blood play important role in reproductive performances. Several studies have indicated that repeat breeding and lowered fertility in cattle may be associated with the imbalance of calcium and phosphorus (Agarwal et al, 1982: Sarma et al, 1984: Awasthi and Kharche, 1987 and Islam et al, 1994). In repeat breeding cows level of serum Phosphorus has been found to be significantly lower (Awasthi and Kharche, 1987).

Administration of HCG at the time of AI has been found to be effective to treat anovulation and delayed ovulation in cattle(Arthur,1971 and Roberts, 1971 : Laing, 1979; Zemjanis,1980:Sandhu and Sing,1992 and Srivastava et al,1998).

Through Deka, 1994 studied certain aspects of ovulatory disturbances and different treatment schedules in repeat breeding crossbred cattle of Assam,it was found overviewing the available literature that no such work was undertaken in the Indigenous cows of West Bengal.

The experimental animals included in the study were repeat breeding Indigenous cows with anovulation, repeat breeding indigenous cows wit delayed ovulation and normal breeding indigenous cows.18 cows were taken into consideration in each of the above three groups totaling 54 cows. The animals under study were belonged to Calcutta Pinjarapole Society, Kanchrapara,24Pgs(N), West Bengal.

Treatment of repeat breeding due to anovulation

A) Treatment with progesterone (Duraprogen)

In the present study progesterone at the dose rate of 25 mg I/M at proestrus was used for treatment of repeat breeding due to anovulation in a total of 6 cows. The idea behind this treatment was that little amount of progesterone with estrogen could hasten ovulatory process in cattle(Hansel and Trimberger,1952; Cole and Cupps, 1969).

Earlier reports indicated that administration of little amount of progesterone after breeding as found to improve the conception rate of repeat breeding cows (Herrick, 1953: Dawsonn, 1954: Jhonson et a.l, 1958 and Hawk et al., 1963).

Results of the present study showed that IM of 25 mg Progesterone during Proestrus was effective in inducing pregnancy in only 1(16.66%) cows, out of a total 6 repeat breeding indigenous cows with anovulation in the subsequent oestrus. On the other hand Panchal et al,1991 and Umakanthan et al., 1995 claimed that progesterone at the dose rate of 250 mg IM improved the conception rate very significantly. The conception rate was 63.64% and 96% respectively. So it may be concluded that Progesterone at the dose rate of 25 mg in the present study was not much effective in treating the repeat breeding with anovulation in Indigenous cows of West Bengal. Winkler, 1973 also reported that progesterone had little or no beneficial effect in the treatment of repeat breeding condition in cattle.

B) Chorionic gonadotrophin (Chorulon)

In the present study Chorulon at the dose rate of 300 IU given I/V at the time of insemination was used for the treatment of repeat breeding due to anovulation in a total of 6cows. However, chorionic gonadotrophin

because of its LH like activity had been recommended for the treatment ofrepeat breeding in cattle by number of workers (Roberts, 1971; Laing, 1979 and Zemjanis, 1980)

Results of the present study indicated that out of 6 repeat breeding indigenous cows with anovulation, 4 (66.66%) showed ovulation in response to IV administration of 3000 IU of Chorionic gonadotrophin at the time of insemination and all 4 (66.66%) conceived. The conception rate recorded in the present study were however, more or less similar to that reported by Sandhu et al., (1992) in repeat breeding cows (67.20%). Mehta et al., (1986) and srivastava et al., (1998) used chorionic gonadotrophin at the dose rate of 1500 IU per animal for treatment of ovulatory disturbance in crossbred cows and heifers and obtained a conception rate as 93.33% and 70% respectively which were comparatively higher than obtained in the present study.Wagh et al., (1991) on the other hand reported that only 16.67% repeat breeding crossbred cows conceived in response to treatment with 5000 IU of LH. This variation in success rate in the treatment of repeat breeding in cows under the present study was definitely anovulation while in the case of cattle investigated by Mehata et al., (1986) and Wagh et al., (1991) the cause was obscure.

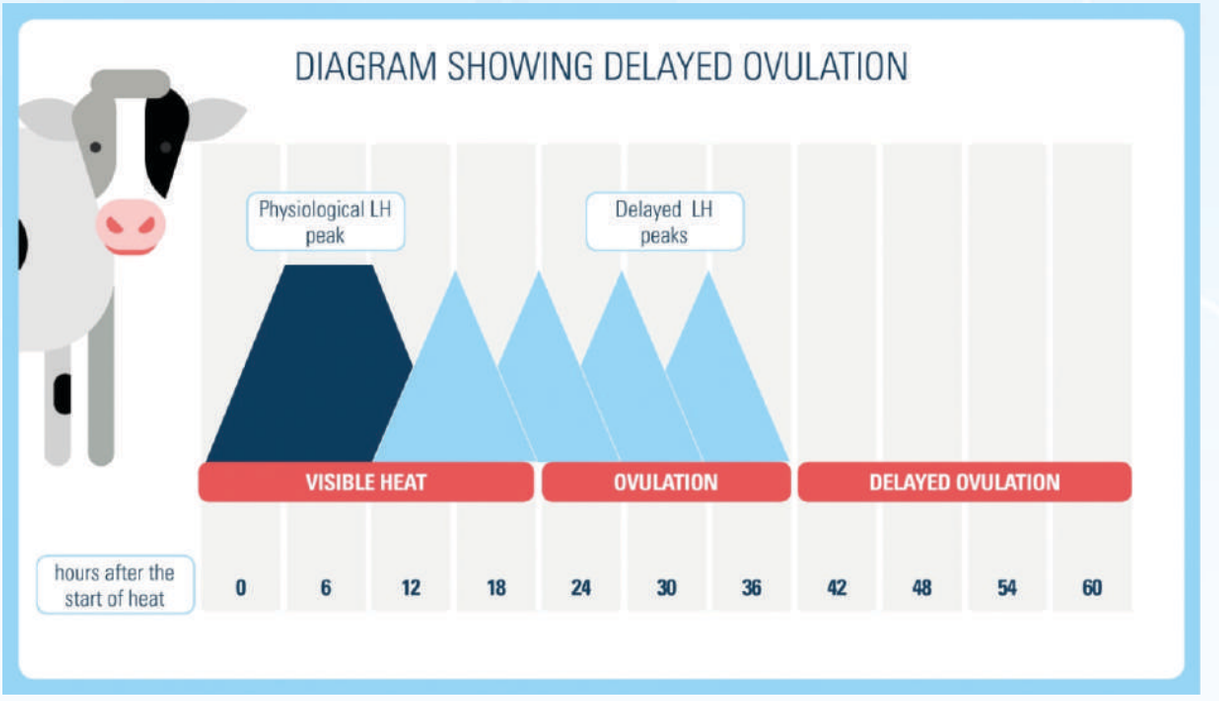
Manual rupture of Graffian Follicle

Result of the present study indicated that manual rupture of the anovulatory follicle during oestrus followed by AI in the subsequent oestrus was a successful treatment of repeat breeding due to anovulation.All 6 repeat breeding anovulatory cows showed normal development of CL following manual rupture of the anovulatory follicle and 50% of them conceived when inseminated in the subsequent oestrus, manual rupture of anovulatory follicle followed by AI in the subsequent oestrus might therefore be recommended as the line of treatment of repeat breeding due to anovulation in Indigenous cows reared by poor farmers who cannot afford to purchase hormonal drugs of high price.

Treatment of repeat breeding due to delayed ovulation

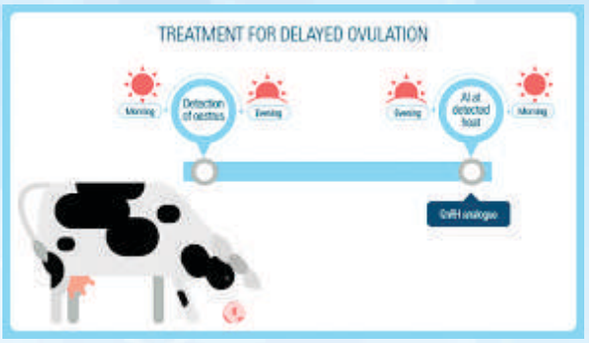
A. Treatment with Progesterone (Duraprogen)

In the present study progesterone 25 mg per animal was administered IM during proestrus in 6 repeat breeding Indigenous cows with delayed ovulation and only 1 (16.66%) cows conceived. On the contrary Mehta et al(1986) using 25 mg of progesterone IM ly at the time of estrus in crossbred cows and heifers with ovulatory disturbances obtained subsequent conception rate of 33.3%.



B. Treatment with chorionic gonadotrophin (Chorulon)

In the present study chorionic gonadotrophin at the dose rate of 3000IU given I/V ly at the time of insemination was used for the treatment of repeat breeding due to delayed ovulation in a total of 6 cows. It was reported that I/V administration of 1000-5000 IU HCG induced ovulation in cattle (Roberts, 1971: Laing, 1979). Out of total 6 animals with delayed ovulation in the present study 4(66.66%) conceived in response to treatment with chorionic gonadotrophin.The conception rate observed in repeat breeding Indigenous cows in the present study were however nearly similar to that reported by Sandhu et al(1992) in repeater cows(67.20%).



C. Manual rupture of graffian follicle followed by AI in the subsequent oestrus

All 6 repeat breeding Indigenous cows with delayed ovulation showed formation of mature CL as revealed on rectal palpation of ovary after 10 th day of oestrus in response to manual rupture of graffian follicle during oestrus. Two (33.33%) conceived when insemination was done in subsequent oestrus. This lower conception rate indicated that manual rupture of graffian follicle during oestrus followed by AI in the subsequent oestrus could not be recommended as the line of treatment for treating of repeat breeding due to delayed ovulation in cows.

Conclusion

Chorionic gonadotrophin (Chorulon) at the dose rate of 3000 IU per animal given intravenously just after AI should be the drug of choice for treatment of anovulation and delayed ovulation in cows. Manual rupture of graffian follicle during oestrus followed by AI at at the subsequent oestrus should be second choice of treatment to combat the anovulatory disturbances of Indigenous cows. More over this treatment will be cheaper and the poor rural farmers will be benefited by this type of treatment as it will not entail much expenditure.

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