Call for Editorial Board Members

As you are well aware that we are a medical and health sciences publishers; publishing peer-reviewed journals and books since 2004.

We are always looking for dedicated editorial board members for our journals. If you completed your master's degree and must have at least five years experience in teaching and having good publication records in journals and books.

If you are interested to be an editorial board member of the journal; please provide your complete resume and affiliation through e-mail (i.e. info@rfppl.co.in) or visit our website (i.e.www.rfppl.co.in) to register yourself online.

Call for Publication of Conference Papers/Abstracts

We publish pre-conference or post-conference papers and abstracts in our journals, and deliver hard copy and giving online access in a timely fashion to the authors.

For more information, please contact:

For more information, please contact:
A Lal
Publication-in-charge
Red Flower Publication Pvt. Ltd.
48/41-42, DSIDC, Pocket-II
Mayur Vihar Phase-I
Delhi – 110 091 (India)
Phone: 91-11-22754205, 45796900
E-mail: info@rfppl.co.in

Free Announcements of your Conferences/Workshops/CMEs

This privilege to all Indian and other countries conferences organizing committee members to publish free announcements of your conferences/ workshops. If you are interested, please send your matter in word formats and images or pictures in JPG/JPEG/Tiff formats through e-mail attachments to sales@rfppl.co.in.

Terms & Conditions to publish free announcements:

1. Only conference organizers are eligible up to one full black and white page, but not applicable for the front, inside front, inside back and back cover, however, these pages are paid.
2. Only five pages in every issue are available for free announcements for different conferences.
3. This announcement will come in the next coming issue and no priority will be given.
4. All legal disputes subject to Delhi jurisdiction only.
5. The executive committee of the Red Flower Publication reserve the right to cancel, revise or modify terms and conditions any time without prior notice.

For more information, please contact:
A Lal
Publication-in-charge
Red Flower Publication Pvt. Ltd.
48/41-42, DSIDC, Pocket-II
Mayur Vihar Phase-I
Delhi – 110 091 (India)
Phone: 91-11-22754205, 45796900
E-mail: info@rfppl.co.in

Win Free Institutional Subscription!

Simply fill out this form and return scanned copy through e-mail or by post to us.

Name of the Institution_____
Name of the Principal/Chairman_____
Management (Trust/Society/Govt./Company)_____
Address 1_____
Address 2_____
Address 3_____
City_____
Country_____
PIN Code_____
Mobile_____
Email_____

We are regular subscriber of Red Flower Publication journals.

Year of first subscription_____

List of ordered journals (if you subscriberd more then 5 titles, please attach separate sheet)

Ordered through

Name of the Vendor	Subscription Year	Direct/subs Yr

Name of the journal for which you wish to be free winner

Terms & Conditions to win free institutional subscription

1. Only institutions can participate in this scheme
2. In group institutions only one institution would be winner
3. Only five institutions will be winner for each journal
4. An institution will be winner only for one journal
5. The free subscription will be valid for one year only (i.e. 1 Jan – 31 Dec)
6. This free subscription is not renewable, however, can be renewed with payment
7. Any institution can again participate after five years
8. All legal disputes subject to Delhi jurisdiction only
9. This scheme will be available to participate throughout year, but draw will be held in last week of August every year
10. The executive committee of the Red Flower Publication reserve the right to cancel, revise or modify terms and conditions any time without prior notice.

I confirm and certify that the above information is true and correct to the best of my knowledge and belief.

Place: Signature with Seal
Date:

Revised Rates for 2020 (Institutional)					
Title of the Journal	**Frequency**	**India(INR) Print Only**	**India(INR) Online Only**	**Outside India(USD) Print Only**	**Outside India(USD) Online Only**
Community and Public Health Nursing	3	6000	5500	469	430
Indian Journal of Agriculture Business	2	6000	5500	469	430
Indian Journal of Anatomy	4	9000	8500	703	664
Indian Journal of Ancient Medicine and Yoga	4	8500	8000	664	625
Indian Journal of Anesthesia and Analgesia	6	8000	7500	625	586
Indian Journal of Biology	2	6000	5500	469	430
Indian Journal of Cancer Education and Research	2	9500	9000	742	703
Indian Journal of Communicable Diseases	2	9000	8500	703	664
Indian Journal of Dental Education	4	6000	5500	469	430
Indian Journal of Diabetes and Endocrinology	2	8500	8000	664	625
Indian Journal of Emergency Medicine	4	13000	12500	1016	977
Indian Journal of Forensic Medicine and Pathology	4	16500	16000	1289	1250
Indian Journal of Forensic Odontology	2	6000	5500	469	430
Indian Journal of Genetics and Molecular Research	2	7500	7000	586	547
Indian Journal of Law and Human Behavior	3	6500	6000	508	469
Indian Journal of Legal Medicine	2	9000	8500	703	664
Indian Journal of Library and Information Science	3	10000	9500	781	742
Indian Journal of Maternal-Fetal & Neonatal Medicine	2	10000	9500	781	742
Indian Journal of Medical and Health Sciences	2	7500	7000	586	547
Indian Journal of Obstetrics and Gynecology	4	10000	9500	781	742
Indian Journal of Pathology: Research and Practice	6	12500	12000	977	938
Indian Journal of Plant and Soil	2	7000	6500	547	508
Indian Journal of Preventive Medicine	2	7500	7000	586	547
Indian Journal of Research in Anthropology	2	13000	12500	1016	977
Indian Journal of Surgical Nursing	3	6000	5500	469	430
Indian Journal of Trauma and Emergency Pediatrics	4	10000	9500	781	742
Indian Journal of Waste Management	2	10000	9500	781	742
International Journal of Food, Nutrition & Dietetics	3	6000	5500	469	430
International Journal of Forensic Science	2	10500	10000	820	781
International Journal of Neurology and Neurosurgery	4	11000	10500	859	820
International Journal of Pediatric Nursing	3	6000	5500	469	430
International Journal of Political Science	2	6500	6000	508	469
International Journal of Practical Nursing	3	6000	5500	469	430
International Physiology	3	8000	7500	625	586
Journal of Animal Feed Science and Technology	2	8300	7800	648	609
Journal of Cardiovascular Medicine and Surgery	4	10500	10000	820	781
Journal of Emergency and Trauma Nursing	2	6000	5500	469	430
Journal of Food Additives and Contaminants	2	6000	5500	430	391
Journal of Food Technology and Engineering	2	5500	5000	430	391
Journal of Forensic Chemistry and Toxicology	2	10000	9500	781	742
Journal of Global Medical Education and Research	2	6400	5900	500	461
Journal of Global Public Health	2	12500	12000	977	938
Journal of Microbiology and Related Research	2	9000	8500	703	664
Journal of Nurse Midwifery and Maternal Health	3	6000	5500	469	430
Journal of Orthopedic Education	3	6000	5500	469	430
Journal of Pharmaceutical and Medicinal Chemistry	2	17000	16500	1328	1289
Journal of Plastic Surgery and Transplantation	2	8000	7500	625	575
Journal of Psychiatric Nursing	3	6000	5500	469	430
Journal of Radiology	2	8500	8000	664	625
Journal of Social Welfare and Management	4	8000	7500	625	586
New Indian Journal of Surgery	6	8500	7500	664	625
Ophthalmology and Allied Sciences	3	6500	6000	508	469
Pediatric Education and Research	4	8000	7500	625	586
Physiotherapy and Occupational Therapy Journal	4	9500	9000	742	703
RFP Gastroenterology International	2	6500	6000	508	469
RFP Indian Journal of Hospital Infection	2	13000	12500	1016	977
RFP Indian Journal of Medical Psychiatry	2	8500	8000	664	625
RFP Journal of Biochemistry and Biophysics	2	7500	7000	586	547
RFP Journal of Dermatology (Formerly Dermatology International)	2	6000	5500	469	430
RFP Journal of ENT and Allied Sciences (Formerly Otolaryngology International)	2	6000	5500	469	430
RFP Journal of Gerontology and Geriatric Nursing	2	6000	5500	469	430
RFP Journal of Hospital Administration	2	7500	7000	586	547
Urology, Nephrology and Andrology International	2	8000	7500	625	586

Terms of Supply:

1. Agency discount 12.5%. Issues will be sent directly to the end user, otherwise foreign rates will be charged.
2. All back volumes of all journals are available at current rates.
3. All journals are available free online with print order within the subscription period.
4. All legal disputes subject to Delhi jurisdiction.
5. Cancellations are not accepted orders once processed.
6. Demand draft/cheque should be issued in favour of "**Red Flower Publication Pvt. Ltd.**" payable at **Delhi.**
7. Full pre-payment is required. It can be done through online (http://rfppl.co.in/subscribe.php?mid=7).
8. No claims will be entertained if not reported within 6 months of the publishing date.
9. Orders and payments are to be sent to our office address as given below.
10. Postage & Handling is included in the subscription rates.
11. Subscription period is accepted on calendar year basis (i.e. Jan to Dec). However orders may be placed any time throughout the year.

Order from

Red Flower Publication Pvt. Ltd., 48/41-42, DSIDC, Pocket-II, Mayur Vihar Phase-I, Delhi - 110 091 (India)
Mobile: 8130750089, Phone: 91-11-45796900, 22754205, 22756995, E-mail: sales@rfppl.co.in, Website: www.rfppl.co.in

Journal of Animal Feed Science and Technology

RED FLOWER PUBLICATION PVT. LTD.

Managing Editor
A. Lal

Publication Editor
Dinesh Kr. Kashyap, Manoj Kumar Singh

Journal of Animal Feed Science and Technology (JAFST) (**ISSN 2321–1628)** is an extraordinary peer-reviewed journal publishing scientific research papers of international interest focusing on animal feeds and their feeding. The all papers in journal are describing research on feed for ruminants and non-ruminants, including all pet and aquatic animals.

Abstracting and Indexing information: ProQuest, USA; Genamics JournalSeek.

Subscription Information

India (One Year**)**

Institutional Rs.8300

ROW (One Year**)**

Insitutional USD648

Payment Methods

• PayPal Instructions for the payment (only for transfer from outside India)
• Payments can be made through our PayPal account at https://www.paypal.com. Our PayPal recipient email address is redflowerppl@gmail.com.
• Bank draft/cashier's order/cheque/demand draft/money order make payable to **Red Flower Publication Private Limited** at Delhi.
• International Bank transfer/bank wire/electronic funds transfer/money remittance/ money wire/telegraphic transfer/telex

Other Information

1. Complete Bank Account No. 604320110000467
2. Beneficiary Name: Red Flower Publication Pvt. Ltd.
4. Bank & Branch Name: Bank of India; Mayur Vihar
6. MICR Code: 110013045
7. Branch Code: 6043
8. IFSC Code: BKID0006043 (used for RTGS and NEFT transactions)
9. Swift Code: BKIDINBBDOS

Send all Orders to: **Red Flower Publication Pvt. Ltd.,** 48/41-42, DSIDC, Pocket-II, Mayur Vihar Phase-I, Delhi – 110 091, India, Phone: 91-11-22754205, 45796900, Fax: 91-11-22754205, E-mail: sales@rfppl.co.in, Website: www.rfppl.co.in

JOURNAL OF ANIMAL FEED SCIENCE AND TECHNOLOGY

July – December 2019
Volume 7 Number 2

Contents

Orignal Article

Journal of Animal Feed Science and Technology
Volume 7 Number 2, July – December 2019
DOI: http://dx.doi.org/10.21088/jafst.2321.1628.7219.1

Efficient *in vitro* Multiplication Protocol for *Vanilla Planifolia* Using Nodal Segments

A Thanga Hemavathy

Assistant Professor (PBG), Department of Pulses, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, 641003, India.

How to cite this article:
A Thanga Hemavathy. Efficient *in vitro* Multiplication Protocol for *Vanilla Planifolia* Using Nodal Segments. Journal of Animal Feed Science and Technology. 2019;7(2):61–64.

Abstract

Vannila planifolia Andrews is one of the most important flavouring herbaceous perennial crop. In vanilla *in vitro* propagation study was conducted to assess the *in vitro* response of various explants in different growth regulators to identify the ideal and suitable medium for primary callus induction and to found out the response of *in vitro* regeneration of plantlets from callus tissue. Three different explants *viz.,* Nodal segment, shoot tip and aerial tip from healthy plants were undertaken for this study. Explants were inoculated in MS medium (Murashige and Skoog 1962) supplemented with BAP, IAA, D-biotin, Calcium pentothenate for primary callus initiation. The growth of multiple shoots was more vigorous on MS medium with 0.2 mg/L of KN and 0.1 mg/L of NAA. MS medium with 1.5 mg/L of IBA and 1.0 mg/L of BA gave good results for rooting. The explant nodal segment gave best response both early shoot induction and higher shooting percentage and also earlier rooting induction with rooting percentage.

Keywords: Micro propagation; Nodal segment; Shooting; Rooting.

Introduction

Vanilla Planifolia Andrews is one of the most important flavouring herbaceous perennial crop. In vanilla the method of propagation is rather slow, labour intensive and time consuming. Moreover, collection of vanilla stem cuttings leads to arrest the growth and development of the mother plants (Ayyappan, 1990). As an alternative approach is tissue culture technique, it is used to selection and rapid multiplication. In India the micro propagation technique had gained momentum to reoccupy the monopoly in vanilla tissue culture at global level. It is able to regenerate the millions of copies to ensure in a decade of time with high yielding with shorter duration, and the tissue culture plants from different explants perform uniformly. Present

Corresponding Author: A Thanga Hemavathy, Assistant Professor (PBG), Department of Pulses, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, 641003, India.

E-mail: hemavathytnau@gmail.com

Received on 13.11.2019; **Accepted on** 04.12.2019

study was conducted to identify the suitable ideal medium for primary culture induction and standardize the method for *in vitro* regeneration of plantlets from callus tissue.

Materials and Methods

In vitro studies were conducted at Agricultural College & Research Institute, Madurai during 2013. The successful *in vitro* culture results depend on the interplay of the plant material (explants), medium in use and the culture environment. Two different explants namely nodal segment and shoot tip are involved in this study. The explants were surface sterilized with 0.1 percent mercuric chloride for 10 minutes followed by washed with distilled water. Then it is rinsed with 70% ethanol for 3 times then it is washed with distilled water. Now the explants were ready for inoculation. The medium contained sucrose and solidified with 0.8% agar, pH of the media was adjusted to 5.7 before autoclaving at 121°C for 20 minutes. Explants were inoculated in MS medium (Murashige and Skoog 1962) supplemented with various combinations of growth regulators for primary callus initiation. Cultures were maintained at 27 ± 2°C temperature, 75% relative humidity and with 16 hours photoperiod.

Statistical analysis

Observations *viz*., callus induction percentage, number of shoots and roots were recorded under *in vitro* condition in the Factorial Completely randomized Block Design (FCRD). Each treatment was replicated three times and ten culture tubes constituted a replication. The percent values were transformed into corresponding arc sine values.

Treatments involved

Shooting

T_1 – Normal MS media + KN 0.2 mg/L + NAA 0.1 mg/L

T_2 – Normal MS media + KN 0.3 mg/L + NAA 0.2 mg/L

T_3 – normal MS media + KN 0.4 mg/L + NAA 0.3 mg/L

Rooting

T_4 – Normal MS media + BAP 1.5 mg/L + IBA 1.0 mg/L

T_5 – Normal MS media + BAP 2.0 mg/L + IBA 2.0 mg/L

T_6 – normal MS media + KN 2.5 mg/L + NAA 3.0 mg/L

Results and Discussion

In the present investigation growth regulator combination of normal MS media + KN 0.4 mg/L + NAA (0.3 mg/L) gave the better results for callus induction and shoot proliferation. The mean value of shooting duration was ranged from 35 to 62 days between the explants. Among the explants tested the duration for shooting was well noticed in nodal segment (Table 1). High percentage of multiple shoot induction ranged from 5 to 70 percent between the explants. High percentage of multiple shoots noticed in nodal segment (70 percent) (Table 2). In the present investigation a relatively high ratio of cytokinin 0.4 mg/L to auxin 0.2 mg/L favoured shoot formation. This is an agreement with Murashige and Skoog (1962)., Rao et al., 1992, 1999 and Mary Mathew et al. (1999).

Table 1: Duration taken for shooting

Hormone concentration	Explants	
	Shoot tip (E_1)	Nodal segment (E_2)
T_1	50	35
T_2	60	50
T_3	62	55
SE d	0.014	0.164
CD (0.05)	0.045	0.52

Table 2: Percentage of callus showing multiple shoots

Hormone concentration	Explants	
	Shoot tip (E_1)	Nodal segment (E_2)
T_1	50	35
T_2	60	50

Hormone concentration	Explants	
	Shoot tip (E_1)	Nodal segment (E_2)
T_3	62	55
SE d		0.520.15
CD (0.05)		1.450.48

The mean for rooting duration ranged from 15 to 40 days between the explants. Among the explants tested the duration for rooting in nodal segment was significantly earlier than the shoot tip. The hormonal combination T_2 – Normal MS media + BAP 2.0 mg/L + IBA (2.0 mg/L) shows better results in roots induction (Table 3). The mean value of root length was ranged from 2.2 to 7.2 cm between the explants. Among the explant long root length was observed in nodal segment at the hormonal combination of T_3 – normal MS media + KN 2.5 mg/L + NAA (3.0 mg/L). A callus with roots will almost never form shoots, as root formation within a callus masks the end of morphogenesis with no possibility of plantlet production unless shoot buds are induced (Kuruvilla 1997). A relatively high ratio of auxin to cytokinin favours root formation (Murashige and Skoog, 1962). Significant differences have been observed in the morphogenetic capacity of calli that were induced at various levels of auxin.

Table 3: Duration for rooting

Hormone concentration	Explants	
	Shoot tip (E_1)	Nodal segment (E_2)
T_4	28	15
T_5	34	22
T_6	–	45
SE d	0.308	0.213
CD (0.05)	0.098	0.678

Table 4: Number of roots per shoot

Hormone concentration	Explants	
	Shoot tip (E_1)	Nodal segment (E_2)
T_4	1.2	2.1
T_5	0.31	0.80
T_6	0.14	0.40
SE d	0.308	0.213
CD (0.05)	0.098	0.678

Conclusion

In vanilla *in vitro* propagation study was conducted to assess the *in vitro* response of various explants in different growth regulators to identify the ideal and suitable medium for primary callus induction and to found out the response of *in vitro* regeneration of plantlets from callus tissue. In the present investigation, the explant nodal segment showed 15 days earlier rooting coupled with higher number of roots/shoots (2:1) than the other explants tested under the treatment KN 0.4 mg/L with NAA 0.2 mg/L. Hence the nodal explants with this hormonal combination will enhance the *in vitro* response of vanilla.

References

1. Ayyappan P. Vanilla a money making venture for ambitious farmers. Kisan world 1990;7:24–26.
2. Kuruvilla JT, Kim K and Sagawa Y. Shoot tip culture of Vanilla. Amer. Orchid Soc. Bull. 1972;41:435–39.
3. Mary Mathew K, Rao YS, Pradip KK, et al. *In vitro* culture systems in Vanilla In: Plant Tissue culture and biotechnology, Emerging Trends (Ed) Kavikishor, P.B. Universities Press (I) ltd, Hyderabad: 1999.pp.171–79.
4. Murashige T and Skoog T. A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiologia of Plant arum. 1962;15:473–97.
5. Rao YS, Mary Mathew K, Madhusoodanan KJ

and Naidu R. Multiple shoot regeneration in Vanilla (*Vanilla planifolia* Andrews). J. Plant. Crops. 1992;21(Supply):351–54.

6. Rao YS, Mary Mathew K, Pradip KK et al. A manual for micro propagation of selected spices. Indian Cardamom Research Institute, Spices Board, Kochi, 1999;25.

Orignal Article

Journal of Animal Feed Science and Technology
Volume 7 Number 2, July – December 2019
DOI: http://dx.doi.org/10.21088/jafst.2321.1628.7219.2

Effect of Replacing Fish Meal Protein By Shrimp Waste Meal Protein With or Without Amino Acids on Serological, Hematological Parameters and Carcass Traits of Broilers

N Mounica[1], JV Ramana[2], D Srinivasa Rao[3], J Suresh[4], P Kavitha[5]

[1]Research Scholar, [2]Controller of Examination, [3]Registrar, [4]Professor, Department of Livestock Production Management [5]Assistant Professor, Department of Animal Nutrition, Sri Venkateswara Veterinary University, Tirupati, Andhra Pradesh 517502, India.

How to cite this article:

N Mounica, JV Ramana, D Srinivasa Rao, et al. Effect of Replacing Fish Meal Protein By Shrimp Waste Meal Protein With or Without Amino Acids on Serological, Hematological Parameters and Carcass Traits of Broilers. Journal of Animal Feed Science and Technology. 2019;7(2):65–71.

Abstract

The present experimental evaluation was to assess the effect of feeding Shrimp waste meal as a replacement for fish meal on serological, hematological parameters and carcass traits in broilers. A growth trial was conducted for 42 days using 375 commercial day old chicks which were distributed randomly into five treatments groups with three replicates of twenty five birds each. In pre-starter diet fish, meal contribution to the dietary crude protein was replaced with shrimp waste meal at 0% (T_1), 20% (T_2), 30% (T_3), T_2 + synthetic lysine and methionine (T_4), T_3 + synthetic lysine and methionine (T_5). In starter and finisher diets the shrimp waste meal protein was added up to the 0% (T_1), 50% (T_2), 100% (T_3), 50% + synthetic lysine and methionine (T_4), 100% + synthetic lysine and methionine (T_5). In pre-starter and starter phases no significant difference was noticed regarding levels of serum total protein (g/dl), albumin (g/dl), globulin (g/dl), glucose (mg/dl) and cholesterol (mg/dl) among treatments. Similarly, in finisher phase there were no significant difference in levels of serum total protein (g/dl), albumin (g/dl) and glucose (mg/dl) among treatments, except the serum cholesterol levels (mg/dl) and globulin levels (g/dl) were found significantly ($p < 0.01$) higher in birds fed T_1 diet than birds fed other diets (T_2, T_3, T_4 and T_5). Non significant differences were noticed among treatment groups regarding RBC count during the three phases of the study. During pre-starter phase the WBC count ($10^3/\mu l$) was significantly higher ($p < 0.01$) in the birds fed with the diets T_2, T_3, T_4 and T_5 when compared to the birds fed with T_1(control diet). During starter and finisher phases the WBC count ($10^3/\mu l$) was significantly higher ($p < 0.01$) in the birds fed with the diets T_2 and T_3 than the birds fed with T_1, T_4 and T_5. During the pre-starter and starter phases. Lymphocyte (%) count was significantly higher ($p < 0.01$) in T_2 and T_3 than in T_1, T_4 and T_5 fed birds. Whereas during the finisher phase there was no significant difference among treatments. At the end of experiment, the live weight gain, hot carcass weight and the dressing percentage were found significantly ($p < 0.01$) higher in birds fed T_4 diet when compared to birds fed other diets. The liver, gizzard and heart weights (g) were found significantly ($p < 0.01$) higher in birds fed T_4, T_2, and T_1 diets when compared to T_3 and T_5 group. The results of the present study depicts that the Protein from FM can be safely substituted up to 30% with the SWM protein in pre-starter and up to 50% in starter and finisher broiler diets along with the supplementation of synthetic amino acids (lysine and methionine).

Keywords: Shrimp waste meal; Fish meal; Amino acids; Serological and hematological parameters; Carcass traits; Broilers.

Corresponding Author: Dr. JV Ramana, Controller of Examination, Department of Animal Nutrition, Sri Venkateswara Veterinary University, Tirupati, Andhra Pradesh 517502, India.

E-mail: jvenkataramana@rediffmail.com

Received on 05.11.2019; **Accepted on** 04.12.2019

Introduction

Poultry industry in India is recognized as an organized and scientifically based industry and potential tool to fight poverty and malnutrition. The potential of poultry industry in alleviating the challenges of low availability of animal protein for human consumption in developing countries is being hampered by high cost of production. The use of agro–industrial by–products have been one of the panaceas for high feed cost in developing countries. One such unconventional protein supplement for broilers is Shrimp Waste Meal (SWM). Shrimp waste can be potentially channeled as a substitute for fish meal in poultry diets. Haematological observations provide valuable information about health of human and animals. According to Afolabi et al., (2010), changes in haematological parameters are often used to determine health status of the body and to know the degree of environmental, nutritional and/or pathological stresses and Maxwell et al., (1990) also stated that blood analysis is a readily available and fast means of assessing clinical and nutritional health status of animals on feeding trials because, ingestion of dietary components has measurable effects on blood composition. Togun et al., (2007) reported that when the haematological values fall within the normal range for the animal, it is an indication that diets not have any adverse effect on haematological parameters during the experimental period. Serum biochemical constituents are positively correlated with the quality of the diet (Brown and Clime, 1972, Adeyemi et al., 2000). Measuring blood metabolites (RBC, WBC, Lymphocyte %), serum constituents (Total protein, Glucose, Cholesterol, Albumin, Globulin) of birds fed SWM can be used as a basis for comparison to fish meal. However, there is paucity of information on the hematology and serum chemistry of the broilers fed with SWM based diet. It is based on this background that the present experimental study was taken up to assess the effect of feeding Shrimp waste meal as a replacement for fish meal on serological, hematological parameters and carcass traits in broilers.

Materials and Methods

Three hundred and seventy five, day old commercial broiler chicks were distributed randomly to five treatments with three replicates of twenty five birds each. The experimental diets in pre-starter phase were prepared by replacing fish meal protein of the basal diet with the Shrimp waste meal protein at 20% level (T_2), 30% level (T_3) and T_4, T_5 diets were prepared by adding synthetic lysine and methionine in T_2 and T_3 diets. In starter and finisher phases five experimental diets were prepared by replacing fish meal protein of the basal diet with the Shrimp waste meal protein at 50% level (T_2), 100% level (T_3) and T_4, T_5 diets were prepared by adding synthetic lysine and methionine in T_2 and T_3 diets. The basal diet T_1 was used as control containing maize, SBM, DORB and 10% fish meal. All diets were iso-nitrogenous and iso-caloric. Experimental diets were formulated as per ICAR, 2013 specifications for broiler diets. During growth trial randomly two birds per replicate in each treatment were slaughtered and blood samples were collected at the end of pre starter, starter and finisher phases from each bird for estimation of blood cell count (RBC, WBC, Lymphocyte %) in whole blood and serum was also separated to estimate serum metabolites. The separated serum was then made clear by centrifuging at 3000 RPM for 10 minutes and transferred to dry, clean ependorf tubes and stored in a refrigerator at (–20°C) for estimation of serum parameters (Total protein, Albumin, Globulin, Glucose, Cholesterol)

Table. 1: Ingredient composition (%) and chemical composition (%) of broiler pre-starter experimental diets

Ingredients	T_1	T_2	T_3	T_4	T_5
Maize	58	57.9	58	57.9	58
Soybean meal	20.55	20.4	20.3	20.34	20.21
Fish meal	10.0	8.0	7.0	8.0	7.0
Shrimp waste meal	0	2.18	3.27	2.18	3.27
De-oiled rice bran	5.0	5.0	4.83	5.0	4.83
Palm oil	3.7	3.77	3.85	3.77	3.85
Mineral mixture*	2.0	2.0	2.0	2.0	2.0
DL-methionine	0.2	0.2	0.2	0.21	0.21
L-lysine	0.55	0.55	0.55	0.6	0.63

Ingredients	T_1	T_2	T_3	T_4	T_5
Feed additives**	+	+	+	+	+
Total	**100**	**100**	**100**	**100**	**100**
Chemical composition					
CP	22.1	22.05	22.0	22.08	22.04
ME (kcal/kg)	3002	2998	2999	2997	3000
Lysine	1.18	1.15	1.13	1.20	1.19
Methionine	0.51	0.51	0.50	0.52	0.51
Ca	1.01	1.02	1.02	1.02	1.02
Available P	0.7	0.66	0.63	0.66	0.63
Cost (Rs/kg)	28.7	28.96	28.89	29.04	29.04

* Contained Ca, 25; P, 15; NaCl, 2.5; Fe, 0.35% and Cu, 100; Mn, 200; Co, 50; I, 100 ppm.

** All diets contained Meriplex® – FDS @ 10g/100 kg : (Each gram contains: Vit-B_1, 8 mg; Vit-B_6, 16 mg Vit-B_{12}, 80 μg; Vit-E_{50}, 80 mg; Niacin, 120 mg; Folic acid, 8 mg; Calcium D Pantothenate, 80 mg, Merivite® –AB_2D_3K @ 10 g/100 kg: (Each gram contains: Vit-A 82,500 IU, Vit-B_2 52 mg, Vit-D_3 1200 IU, Vit-K 10 mg, Calcium 166 mg, Phosphate 395 mg) and Cosmodot @ 50g/100 kg: (3–5, Dinitro-O-Toluamide: 25 percent W/W)

Table 2: Composition of broiler starter and finisher experimental diets

Ingredient	T_1		T_2		T_3		T_4		T_5	
	Starter	Finisher	Starter	Finisher	Starter	Finisher	Starter	Finisher	Starter	Finisher
	Ingredient composition (%)									
Maize	59	65	60.25	65	59.51	64.81	60.25	65	59.77	65.24
Soybean meal	19.3	14	19.5	14.2	19.8	14.7	19.39	14.06	19.3	14
Fish meal	10	10	5	5	0	0	5	5	0	0
Shrimp	0	0	5.45	5.45	10.89	10.89	5.45	5.45	10.89	10.89
De-oiled rice bran 5 4.8	5	4.8	3	3.65	2.5	2.5	3.0	3.65	2.5	2.5
Palm oil	4.1	3.8	4.2	4.3	4.7	4.7	4.2	4.3	4.7	4.7
Mineral mixture*	2	2	2	2	2	2	2	2	2	2
DL-methionine	0.17	0.1	0.17	0.1	0.17	0.1	0.18	0.11	0.2	0.13
L-lysine 0.43 0.3	0.43	0.3	0.43	0.3	0.43	0.3	0.53	0.43	0.64	0.54
Feed additives** + +	+	+	+	+	+	+	+	+	+	+
Total	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**1.00**
	Chemical composition (%) DM Basis									
CP	21.5	19.55	21.49	19.5	21.5	19.5	21.5	19.6	21.5	19.5
ME (kcal/kg)	3049	3096	3049.3	3102	3048	3099	3047	3099	3049.2	3098
Lysine	1.09	0.92	0.99	0.83	0.99	0.74	1.01	0.93	1.01	0.92
Methionine	0.48	0.4	0.46	0.39	0.4	0.37	0.47	0.4	0.47	0.4
Ca	1.01	1.07	1.01	1.09	1.02	1.01	1.01	1.08	1.01	1.09
Available P	0.7	0.69	0.58	0.57	0.46	0.45	0.58	0.57	0.46	0.45
Cost (Rs/kg)	28.39	26.39	28.30	26.53	28.49	26.71	28.53	26.82	29.06	27.56

* Contained Ca, 25; P, 15; NaCl, 2.5; Fe, 0.35% and Cu, 100; Mn, 200; Co, 50; I, 100 ppm.

** All diets contained Meriplex® – FDS @ 10 g/100 kg : (Each gram contains: Vit-B_1, 8 mg; Vit-B_6, 16 mg Vit-B_{12}, 80 μg; Vit-E_{50}, 80 mg; Niacin, 120 mg; Folic acid, 8 mg; Calcium D Pantothenate, 80 mg, Merivite® – AB_2D_3K @ 10 g/100 kg: (Each gram contains: Vit-A 82, 500 IU, Vit-B_2 52 mg, Vit-D_3 1200 IU, Vit-K 10 mg, Calcium 166 mg, Phosphate 395 mg) and Cosmodot @ 50g/100 kg: (3–5, Dinitro-O-Toluamide: 25 percent W/W)

Serum total protein was estimated calorimetrically using diagnostic kit (M/s Span diagnostics limited) by Biuret method (Varley et al., 1980). Serum albumin was estimated calorimetrically by using diagnostic kit (M/s Robonic (India) Pvt. Ltd., as per the bromocresol green method (Doumas et al., 1971). The serum globulin values were calculated by subtracting the values of serum albumin from the corresponding values of total protein. Serum glucose was estimated calorimetrically using diagnostic kit (M/s Robonic (India) PVT. LTD.,) by enzymatic GOD/POD method. Serum cholesterol was estimated calorimetrically by using diagnostic kit (M/s Robonic (India) PVT. LTD.,) by enzymatic

method of Allian, (1974).

At the end of experiment, two birds from each replicate and thus a total of six birds per each treatment were randomly chosen, weighed and slaughtered to study carcass characteristics. The liver, gizzard and heart were collected and weighed and the percentages were calculated on live weight basis. All the data obtained in this experiment was subjected to analysis of variance (Snedecor and Cochran, 1994).

Results and Discussion

Serological parameters

No significant differences were observed in serum total protein content (Table 3, 4 and 5) among treatment groups during pre-starter, starter and finisher phases but, there was a slight increase in the serum total protein content in the groups fed with SWM diets when compared to control. The study of Li et al., (2007) well supported the findings of present study that broilers supplemented with chito-oligosaccharide at 100 mg/kg level than other treatment birds have higher serum total protein content. Jabbal et al., (1998); Wang et al., (2003); Tang et al., (2005) also claimed that there was an increase in the level of serum total protein in pigs supplemented with chito-oligosaccharides.

There was no significant difference in serum albumin values among treatment groups during pre-starter, starter and finisher phases (Table 3, 4 and 5). The results are in consonance with the findings of Olayemi, (2001). Similarly, non-significant differences were noticed in serum globulin contents of different treatments during pre-starter and starter phases while, the values differed significantly ($p < 0.01$) in finisher phase.

During pre-starter and starter phases non significant differences were observed in serum cholesterol levels among different treatments (Table 3, 4 and 5, Fig. 1). Significant ($p < 0.01$) reduction in the levels of cholesterol was noticed in the finisher phase with the increase in the level of SWM inclusion and the values ranged from 111.26 mg/dl (T_3) to 120.85 mg/dl (T_1). The observations of the present study are in harmony with the several earlier reports of Li et al., (2007), Kobayashi and Itoh (1991), Razdan and Patterson (1994) who reported the beneficial effect of chito-oligosaccharides present in SWM in reducing blood cholesterol levels. On contrary, Abiodun Adeyeye et al., (2014) reported that the levels of serum cholesterol increased as the level of SWM substitution increased in turkey poults.

During the experimental period, irrespective of the growth phase no significant differences were observed among different treatment groups regarding serum glucose levels (Table 3, 4 and 5). The results of present study were in conformity with the findings of Olayemi, (2001) who reported that there was no significant difference among dietary treatments.

Table. 3: Effect of supplementation of shrimp waste meal on serum parameters during pre starter phase (At the end of 2 weeks of age)

Treatment	Total Protein^{NS} (g/dl)	Albumin^{NS} (g/dl)	Globulin^{NS} (g/dl)	Cholesterol^{NS} (mg/dl)	Glucose^{NS} (mg/dl)
T_1	4.08 ± 0.11	2.56 ± 0.06	1.52 ± 0.07	112.99 ± 0.59	379.02 ± 2.29
T_2	4.26 ± 0.05	2.58 ± 0.05	1.68 ± 0.07	116.0 ± 0.58	378.03 ± 0.99
T_3	4.25 ± 0.07	2.72 ± 0.02	1.52 ± 0.06	113.44 ± 1.04	376.52 ± 0.81
T_4	4.36 ± 0.06	2.7 ± 0.02	1.66 ± 0.06	114.34 ± 0.85	374.83 ± 0.94
T_5	4.56 ± 0.05	2.64 ± 0.04	1.72 ± 0.08	115.65 ± 1.08	371.5 ± 0.76

^{ab} Values in a row not sharing common superscripts differ significantly **($p < 0.01$), NS-Non-significant

Table 4: Effect of supplementation of shrimp waste meal on serum parameters during starter phase (At the end of 4 weeks of age)

Treatment	Total Protein^{NS} (g/dl)	Albumin^{NS} (g/dl)	Globulin^{NS} (g/dl)	Cholesterol^{NS} (mg/dl)	Glucose^{NS} (mg/dl)
T_1	4.43 ± 0.06	2.56 ± 0.08	1.93 ± 0.09	116.43 ± 1.05	285.5 ± 2.52
T_2	4.50 ± 0.14	2.46 ± 0.03	2.03 ± 0.15	118.53 ± 0.9	284.33 ± 3.45
T_3	4.56 ± 0.1	2.75 ± 0.04	1.81 ± 0.11	118.36 ± 1.7	284.83 ± 2.72
T_4	4.71 ± 0.13	2.58 ± 0.06	2.13 ± 0.13	116.58 ± 1.38	284.16 ± 3.78
T_5	4.35 ± 0.1	2.57 ± 0.08	1.77 ± 0.09	117.51 ± 0.68	284.21 ± 1.05

NS- Non-significant

Table 5: Effect of supplementation of shrimp waste meal on serum parameters during finisher phase (At the end of 6 weeks of age)

Treatment	Total Protein^{NS} (g/dl)	Albumin^{NS} (g/dl)	Globulin** (g/dl)	Cholesterol** (mg/dl)	Glucose^{NS} (mg/dl)
T_1	4.58 ± 0.08	2.68 ± 0.04	2.06^{a} ± 0.19	120.85^{a} ± 0.84	373.75 ± 10.9
T_2	4.63 ± 0.06	2.75 ± 0.02	1.71^{ab} ± 0.13	113.65^{bc} ± 1.64	383.22 ± 2.82
T_3	4.31 ± 0.13	2.63 ± 0.02	1.41^{b} ± 0.08	111.26^{c} ± 1.75	386.36 ± 1.73
T_4	4.55 ± 0.12	2.58 ± 0.06	1.5^{b} ± 0.16	115.58^{b} ± 1.38	380.43 ± 2.58
T_5	4.28 ± 0.1	2.81 ± 0.03	1.61^{b} ± 0.07	113.78^{bc} ± 1.28	386.23 ± 1.93

^{abc} Values in a row not sharing common superscripts differ significantly **($p < 0.01$), NS- Non-significant

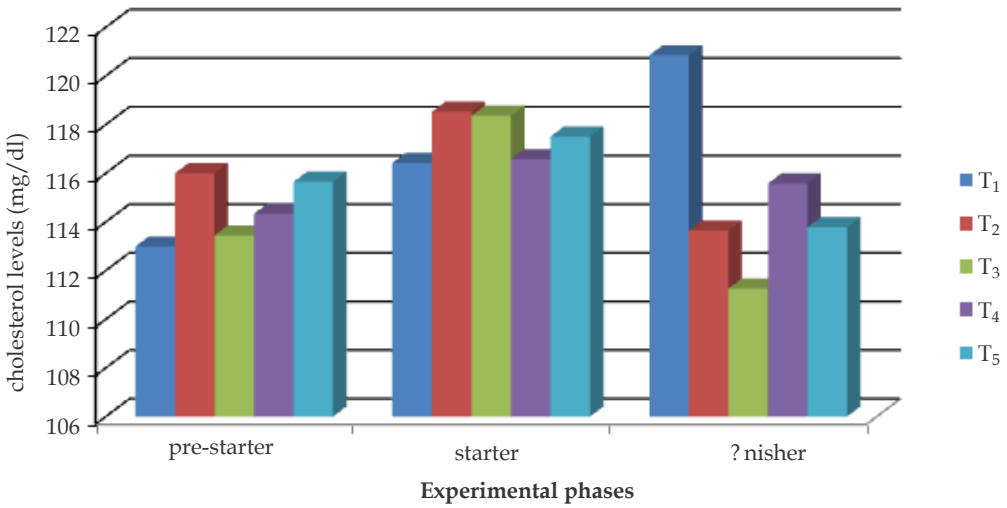

Fig. 1: Effect of supplementation of shrimp waste meal on cholesterol (mg/dl) of Broilers

Hematological Parameters

RBC count did not differ significantly among the treatments in all the phases of Experimental period (Table 6). Significant ($p < 0.01$) differences were observed in WBC count and the values were higher in the birds supplemented with SWM (Table 7). During pre-starter and starter phases higher ($p < 0.01$) lymphocyte (%) was observed in birds fed T_2 and T_3 diets than in other treatments (Table 8). But, in finisher phase no significant difference was observed in lymphocyte (%) among treatments.

Increase in WBC (Meng et al., 2010), RBC (Zhou et al., 2009) were reported due to feeding of chito-oligosaccharide in poultry diets. Chen et al., (2009) reported that supplementation of 5 g/kg chito-oligosaccharide did not affect the WBC, RBC count and lymphocyte (%).

Table 6: Effect of supplementation of shrimp waste meal on RBC count ($10^6/\mu l$)

Phases	T_1	T_2	T_3	T_4	T_5
Pre-starter ^{NS}	2.20 ± 0.05	2.29 ± 0.12	2.16 ± 0.08	2.39 ± 0.2	2.47 ± 0.24
Starter ^{NS}	3.10 ± 0.31	3.38 ± 0.14	3.35 ± 0.16	3.61 ± 0.07	3.08 ± 0.18
Finisher ^{NS}	2.36 ± 0.14	2.55 ± 0.21	2.59 ± 0.14	2.87 ± 0.07	2.71 ± 0.07

NS- Non-significant

Table 7: Effect of supplementation of shrimp waste meal on WBC count ($10^3/\mu l$)

Phases	T_1	T_2	T_3	T_4	T_5
Pre-starter**	27.65^{b} ± 0.34	29.6^{a} ± 0.22	29.86^{a} ± 0.20	29.22^{a} ± 0.25	29.12^{a} ± 0.35
Starter**	29.4^{c} ± 0.17	30.26^{a} ± 0.03	30.07^{ab} ± 0.09	29.79^{b} ± 0.06	29.9^{b} ± 0.09
Finisher**	29.48^{c} ± 0.23	30.22^{a} ± 0.06	29.94^{ab} ± 0.12	29.82^{bc} ± 0.05	29.82^{bc} ± 0.05

^{abc} Values in a row not sharing common superscripts differ significantly **($P < 0.01$)

Table. 8: Effect of supplementation of shrimp waste meal on lymphocyte (%)

Phases	T_1	T_2	T_3	T_4	T_5
Pre-starter**	$56.68^{c} \pm 0.27$	$60.04^{ab} \pm 0.55$	$61.17^{a} \pm 0.12$	$58.7^{b} \pm 1.11$	$58.16^{bc} \pm 0.68$
Starter**	$57.47^{b} \pm 0.31$	$58.89^{a} \pm 0.28$	$58.31^{ab} \pm 0.32$	$57.43^{b} \pm 0.3$	$57.63^{b} \pm 0.39$
Finisher^{NS}	68.21 ± 0.6	67.25 ± 0.83	68.75 ± 0.57	68.17 ± 0.37	67.63 ± 0.4

^{abc}Values in a row not sharing common superscripts differ significantly **($p < 0.01$), NS- Non-significant

Carcass characteristics

The live weight ($p < 0.01$) at slaughter, hot carcass weight ($p < 0.01$) and dressing (%) ($p < 0.01$) were higher ($p < 0.01$) in birds fed on T_4 and lower weight gains were observed with T_3 and T_5 diets (Table 9). The better performance of the birds fed on T_4 diet might be due to improved utilization of SWM protein with the synthetic amino acids supplementation. On contrary, lower performance of the birds fed on T_3 and T_5 diets might be due to decreased utilization of nutrients at higher levels of chitin in the diets with the complete replacement of FM protein with SWM protein. Hector and Lourdes, (2005) and Fanimo et al. (1996) also reported decrease in live weight and carcass % with increase in SWM in the diet.

The liver, gizzard and heart weights at slaughter differed significantly ($p < 0.01$) and higher weights were noticed in the birds fed on T_4 diets while, lower weight gains were observed with T_3 and T_5 diets. In contrary the findings of Okonkwo et al. (2012), Agunbiade et al. (2004), Olayemi, (2001) and Mahata et al. (2008) showed non significant effect of feeding different levels of shrimp waste on liver, gizzard and heart weights of broilers.

Table 9: Effect of supplementation of shrimp waste meal on carcass characteristics of broilers

Treatment	Live wt.** (kg)	Hot carcass wt.** (kg)	Dressing ** (%)	Liver wt.** (g)	Gizzard wt.** (g)	Heart wt.** (g)
T_1	$2.09^{b} \pm 0.03$	$1.36^{ab} \pm 0.02$	$65.51^{c} \pm 0.20$	$45.86^{a} \pm 0.99$	$36.83^{b} \pm 0.90$	$9.98^{ab} \pm 0.25$
T_2	$2.08^{b} \pm 0.04$	$1.39^{ab} \pm 0.03$	$66.83^{b} \pm 0.35$	$46.01^{a} \pm 0.56$	$39.72^{a} \pm 0.56$	$9.69^{abc} \pm 0.35$
T_3	$1.81^{c} \pm 0.06$	$0.98^{c} \pm 0.18$	$63.51^{d} \pm 0.12$	$43.38^{b} \pm 0.55$	$34.58^{bc} \pm 1.32$	$9.27^{bc} \pm 0.08$
T_4	$2.21^{a} \pm 0.04$	$1.49^{a} \pm 0.03$	$67.68^{a} \pm 0.13$	$46.78^{a} \pm 0.63$	$40.02^{a} \pm 0.61$	$10.27^{a} \pm 0.39$
T_5	$1.90^{c} \pm 0.05$	$1.21^{bc} \pm 0.04$	$64.32^{e} \pm 0.2$	$44.95^{ab} \pm 0.64$	$34.07^{c} \pm 0.57$	$8.96^{c} \pm 0.1$

^{abcd} Values in a row not sharing common superscripts differ significantly **($p < 0.01$)

Conclusion

On the basis of present findings it can be concluded that the shrimp waste meal can be utilized as a promising alternative protein source to fish meal in broiler diets and the protein from FM can be safely substituted up to 30% with the SWM protein in pre-starter and up to 50% in starter and finisher broiler diets for good economic returns and productive performance. Supplementation of synthetic lysine, methionine improved the utilization of shrimp waste meal protein.

References

1. Abiodun Adeyeye, Olutosin Odugwa, Oladele Oso, et al. Haematology and serum biochemistry of turkey poults fed shrimp waste meal based diet. Tropentag, September 17–19, 2014, Prague, Czech Republic.
2. Adeyemi OA, Fashina OE and Balogun MO. Utilization of Full-Fat Jatropha Seed in Broiler Diet: Effect on Haematological Parameters and BloodChemistry. In: Proc 25th Annual Conference Nig SocAnim Prod (NSAP) 19th–23rd March, 2000, Umudike. 2000.pp.108–09.
3. Afolabi KD, Akinsoyinu AO, Olajide R, et al. Haematological parameters of the Nigerian local grower chickens fed varying dietary levels of palm kernel cake. 2010.p.247. Proceedings of 35th Annual Conference of Nigerian Society for Animal Production
4. Agunbiade JA, Tolorunji BO and Awojobi HA. Shrimp waste meal supplementation of cassava products based diet fed to broiler chickens. Niger. J. Anim. Prod. 2004;2:182–83.
5. Allian. In vitro enzymatic method of cholesterol estimation. Clinical Chemistry, 1974;26(13):1775.
6. Brown JA and Clime TR. Nutrition and Haematological values. J Anim Sci, 1972;35:211–18.
7. Chen HC, Chang CC, Mau WJ et al. Evaluation of N-acetyl chito- oligosaccharides as the main carbon

sources for the growth of intestinal bacteria. FEMS Microbiol. Lett. 2002;209:53–56.

8. Doumas B, Wabson W and Biggs H. Albumin standards and measurement of serum with bromo cresol green. Clin.Chem. Acta, 1971;31:87.
9. Fanimo AO, Mudama E, Umukoro TO et al. Substitution of shrimp waste meal for fish meal in broiler chicken rations. Trop. Agric. (Trinidad) 1996;73:201–205.
10. Hector L. Santiago and Lourdes I Maldonado. The effect of using shrimp meal in broiler diets on live performance and carcass traits. Proceedings in under-graduate research programme. 2005.
11. ICAR. Nutrient requirements of poultry. ICAR publications, New Delhi. 2013.
12. Jabbal GI, Fisher AN, Rappouli R, et al. Stimulation of mucosal and systemic antibody responses against Bordetella pertussis filamentous haemagglutinin and recombinant pertussis toxin after nasal administration with chitosan in mice. Vaccine. 1998;16:2039–046.
13. Kobayashi S and Itoh H. Effect of dietary chitin and chitosan on growth and abdominal fat deposition in chicks. Jpn. Poult. Sci. 1991;28:88–94.
14. Li X J, Piao XS, Kim SW, et al. Effects of Chito-oligosaccharide supplementation on performance, nutrient digestibility and serum composition in broiler chickens. Poult. Sci. 2007;86:1107–14.
15. Mahata ME, Dharma A, Ryanto I et al. Effect of substituting shrimp waste hydrolysate of Peneus merguensis for fish meal in Broiler. Pak. J. Nut. 2008;7(6):806–10.
16. Maxwell MH, Robertson GW, Spence S, et al. Comparison of haematological values of restricted and ad libitum feeding in domestic fowl, Blood characteristics. Brit. Poult. Scl. 1990;31(3):407–13.
17. Meng QW, Yan L, Ao X, et al. Effect of chito-oligosaccharide supplementation on egg production, nutrient digestibility, egg quality and blood profiles in laying hens. Asian-Australian. J. Anim. Sci., 2010;23:1476–81.
18. Okonkwo AC, Apkan IP and Issac LJ. Performance and carcass characteristics of finisher broilers fed shrimp waste meal. Agricul. J. 2012;7(4):270–72.
19. Olayemi Olugbenga Victor. The feeding value of Shrimp waste meal based diet for finisher broilers. A Project report submitted to the college of Animal science and Livestock production. University of Agriculture Abeokuta, Ogun State -Nigeria. 2001.
20. Razdan A, Pettersson D, Effect of chitin and chitosan on nutrient digestibility and plasma lipid concentration in broiler chickens. British Journal of Nutrition. 1994;72:277–88.
21. Snedecor GW and Cochran WS. Statistical Methods. 8th Edition. Oxford and IB publishing Co., Kolkata. 1994.
22. Tang ZR. Yin YL, Nyachoti CM, et al. Effect of dietary supplementation of chitosan and galacto-mannan-oligosaccharide on serum parameters and the insulin-like growth factor-I mRNA expression in early-weaned piglets. Domest. Anim. Endocrinol, 2005;28:430–41.
23. Togun VA, Farinu GO, Oyebiyi OO, et al. Comparative study of the effect of dietary replacement of 15% maize offal with pigeon pea (Cajanus cajan) grain or leaf meal on performance of weaners, rabbits. Proc of 32nd Annual Conf of the Nig Soc for Anim Prod. 2007;217–219.
24. Varley H, Gowenlock AH and Bell M. Practical Clinical Biochemistry, 5th Edition, William Hienemann Books Ltd. London. 1980.p.550.
25. Wang XW, Du YG, Bai XF et al. The effect of oligo chitosan on broiler gut flora, microvilli density, immune function and growth performance. Acta Zoonutr. Sin. 2003;15:32–45.
26. Zhou TX, Chen YJ, Yoo JS, et al. Effects of chito oligosaccharide supplementation on performance, blood characteristics, relative organ weight and meat quality in broiler chickens. Poult. Sci. 2009;88:593–600.

STATEMENT ABOUT OWNERSHIP AND OTHER PARTICULARS
"Journal of Animal Feed Science and Technology" (See Rule 8)

1. Place of Publication	:	Delhi
2. Periodicity of Publication	:	Half-yealy
3. Printer's Name	:	**Dinesh Kumar Kashyap**
Nationality	:	Indian
Address	:	3/259, Trilokpuri, Delhi-91
4. Publisher's Name	:	**Dinesh Kumar Kashyap**
Nationality	:	Indian
Address	:	3/259, Trilokpuri, Delhi-91
5 Editor's Name	:	**Dinesh Kumar Kashyap**
Nationality	:	Indian
Address	:	3/259, Trilokpuri, Delhi-91
6. Name & Address of Individuals	:	**Red Flower Publication Pvt. Ltd.**
who own the newspaper and particulars of	:	41/48, DSIDC, Pocket-II
shareholders holding more than one per cent of the total capital		Mayur Vihar, Phase-1, Delhi-91

I, **Dinesh Kumar Kashyap**, hereby declare that the particulars given above are true to the best of my knowledge and belief.

Sd/-
(Dinesh Kumar Kashyap)

Review Article

Journal of Animal Feed Science and Technology
Volume 7 Number 2, July – December 2019
DOI: http://dx.doi.org/10.21088/jafst.2321.1628.7219.3

Pesticide Residues in Animal Feed: Status, Safety and Scope

Atul Kumar[1], Ankaj Thakur[2], Vaishali Sharma[3], Shubham Koundal[4]

[1]Assistant Professor, [3,4]Post Graduate Scholar, Department of Veterinary Public Health and Epidemiology, [2]Assistant Professor, Department of Instructional livestock Farm Complex, Dr. G.C. Negi College of Veterinary and Animal Sciences, CSK Himachal Pradesh Agricultural University, Palampur, Himachal Pradesh 176062, India.

How to cite this article:
Atul Kumar, Ankaj Thakur, Vaishali Sharma, et al. Pesticide Residues in Animal Feed: Status, Safety and Scope. Journal of Animal Feed Science and Technology. 2019;7(2):73–80.

Abstract

Rapid increase in population has undoubtedly put enormous pressure on the food production system across the globe. However, to meet this ever increasing food demand and for controlling the pests and vectors, there has been extensive usage of chemicals especially pesticides. Although, pesticides finds an important place in intensive agriculture for enhancing the crop yield and storage; and in animal husbandry for preventing pest infestations but their unregulated and indiscriminate use is a serious health concern. Unfortunately, the rampant use of pesticides poses a potential health risks to humans, animals, and environment as well as to non-target species accruing to biodiversity loss, emergence of resistant pests and other ecological imbalances. Therefore, various steps has been taken to curb their haphazard use and many governments are now imposing restrictions on their usage. But, numerous formulations are still in use, both in agricultural and domestic settings; thereby leading to contamination of natural resources and risks for future generations. The present review focuses on various aspects of pesticides usage in agricultural and animal husbandry practices, their impact on health and the possible alternatives to their use.

Keywords: Pesticides; Animal feed; Health impacts; Environmental health; Consumption pattern.

Introduction

Maintaining balance between increasing population and supply of sufficient food is a major challenge for most of the developing nations, including India. Although, the green revolution enhanced the global food production by many folds during 1966–2000 but the usage of chemical pesticides to achieve these goals also posed some serious threats.[1,2] Since, most of the pesticides used are toxic and have raised a number of environmental worries, including human and

Corresponding Author: Atul Kumar, Assistant Professor, Department of Veterinary Public Health and Epidemiology, Dr. G.C. Negi College of Veterinary and Animal Sciences, CSK Himachal Pradesh Agricultural University, Palampur, Himachal Pradesh 176062, India.

E-mail: dratul9@gmail.com

Received on 28.10.2019; **Accepted on** 28.11.2019

animal health hazards, therefore, these potentially toxic chemicals have proved to be major threat to 'One Health'. One health is a collective approach dealing with human, animal and environmental health. As per World Health Organization (2018), pesticides are chemical compounds that are used to kill pests, including insects, rodents, fungi and unwanted plants (weeds). It covers a broad variety of compounds like insecticides, fungicides, herbicides, rodenticides, molluscicides, nematicides, plant growth regulators and others. Pesticides used in agriculture not only protect the crops from being damaged but also increases their yield. Use of pesticides dates back to the times of ancient Romans where people used to burn sulphur for killing pests and used salts and ashes for controlling weeds.[3] The Rig Veda also mentioned earliest record of using poisonous plants for controlling pests. During 15th–17th century, substances like arsenic, mercury, lead and tobacco leaves extract were being used in crops to kill pests. The 19th and 20th century period witnessed the discovery of various effective and inexpensive pesticides such as Aldrin, DDT, Dieldrin, BHC, 2, 4-D, Chlordane and Endrin.[3,4] Although, in 1960s, following the publication of 'Silent Springs' by Rachel Carson, use of DDT was recognized as a threat to biodiversity and has now been restricted for use in many countries including India. But, worldwide production of pesticides has increased exponentially at a rate of about 11% annually, from 0.2 million tons in 1950s to more than 5 million tons by 2000.[5] Approximately, 2 million tonnes of more than 800 different kinds of pesticides are used every year worldwide out of which India accounts for only 3.75%.[6]

The main purpose behind the introduction of pesticides was to prevent and control insect pests and spread of diseases in the field crops and of course controlling the vector borne disease transmission. Owing to the use of persistent pesticides in crops, there is always a risk of low levels of their residues occurring in animal feed. Therefore, animal feed, whether in the form of commercially available materials or from natural grazing on grass or straw, provides the main exposure route for animals to environmental contaminants and pesticides. The consumption of contaminated animal feed and fodder by the food producing animals further leads to occurrence of their residues in animal products like milk, meat and eggs. Unless the residues are managed at the pre/post-harvest stages or during the storage of animal feeds, it is very difficult to prevent contamination of animal products. Finally, these residues can be transferred to humans via the food chain leading to long-term human health implications. Therefore, food is the major source of human exposure to various contaminants and thus the concerns regarding food safety especially for foods of animal origin are increasing worldwide.[7] Most of the pesticide residue-monitoring programmes have focused on food crops, fruits, and vegetables, products of animal origin such as milk, meat and eggs. However, concerning the presence of residues in animal feed and fodder, there are few reports which highlight the status of pesticide residues in feed and foddersamples from India (Table 1).

Table 1: Pesticide residues reported in various animal feeds and fodder from India

Region	Sample type (*n*)	Method of detection	Detected pesticides (Concentrations in ppm)	References
India (Uttrakhand)	Fodder grasses	Gas-liquid chromatography	∑ –HCH (0.156 – 0.574) ∑ –DDT (0.164 – 0.631)	[8]
India (Andhra Pradesh)	Paddy straw	–	∑ -HCH: ND – 1.92 DDE: ND – 6.26	[9]
India (Punjab)	Wheat straw	GC-ECD	BHC = 0.060 ∑ –DDT = 0.048	[10]
India (Punjab)	Wheat straw	–	∑ –HCH= 0.02 – 0.18 ∑ –DDT = 0.05–10.94	[11]
India (Uttar Pradesh)	Cattle feed (32)	GC-ECD	∑ –DDT (0.236) Endosulfan I (0.029) Endrin (0.020) Aldrin (0.156)	[12]
India (Delhi)	Animal feed (12)	GLC	∑ DDT (ND – 0.251) ∑ HCH (0.007 – 1.86)	[13]
India (Punjab)	Animal Feed (31)	–	DDT, Endosulfan, HCH, Malathion,	[14]
India (Uttrakhand)	Rice Straw	–	∑ –HCH (0.002 – 0.117) ∑–DDT (ND – 0.05)	[15]

Region	Sample type (*n*)	Method of detection	Detected pesticides (Concentrations in ppm)	References
India (Assam)	Concentrate feed and fodder (15)	–	Lindane (0.025 – 0.041) β-endosulfan (0.028) Endosulfansulphate (0.045 – 0.049)	[16]
India (Punjab)	Poultry feed	GC-ECD	Mean HCH (0.65), $\sum$ –DDT (0.91), Endosulphan (0.42) Heptachlor epoxide (0.02)	[17]
India (Haryana)	Animal Feeds	–	pp-DDT (0.007 ± 0.005)	[18]
India (Uttar Pradesh)	Animal Feed stuffs (533)	GC-ECD	$\sum$ –HCH (0.01 – 0.306), $\sum$ -DDT (0.016 – 0.118), Endosulfan (0.009 – 0.237)	[19]
India (Haryana)	Dry and Green Fodder	GC-ECD	$\sum$–OCP (1.1 – 1.2)	[20]
India (Andhra Pradesh)	Poultry Feed		HCH, DDT, Aldrin, Carbendazine and Thiram	[21]
India (Punjab)	Fodder (106)	GC-ECD/FTD	HCH, DDT, endosulfan, cypermethrin, deltamethrin, chlorpyrifos and monocrotophos	[22]
India (Uttrakhand)	Feed and fodder samples (40)	HPLC	α endosulphan (0.402) β endosulphan: (0.147) Endosulphansulphate (0.373)	[23]
India (Haryana)	Poultry Feed (50)	GC-ECD	$\sum$ HCH (618.28) $\sum$ Endosulfan (135.63)	[24]
India (Telangana)	Fodder samples (48)	GC-ECD/PFD	Phorate (0.01 – 0.561)	[25]

Pesticides Consumption Patterns in India and worldwide

Over the last 60 years, farmers have achieved major progress in foodstuff production via the application of pesticides.[26] Pesticides are used in agriculture to control pests, weeds and have contributed to considerable increase in global food production through increasing yield. Reduction of pest activities and agricultural losses at a reasonable amount improved crop yield and thus ensure reliable supplies of agricultural produce at affordable prices to consumers. Also, pesticides improves the cosmetic appeal and quality of the final produce which is important for buyers. When used properly pesticides improve nutritional value of food and safety.[27] Pesticides can be considered as a productive, labor-saving, and effective tool for pest management. According to the US Environmental Protection Agency (US EPA), pesticides proved to be effective in controlling disease organisms too. They can protect the health of humans and animals by controlling insect and rodent populations which are responsible for transmission of many infectious diseases. Use of insecticides to control the insects being considered the effective and only practical way to reduce or stop spread of deadly diseases such as malaria, resulting in an estimated 5000 deaths every day.[28] Approximately, 20 major diseases in India have been brought under control by the application of pesticides; major amongst them are malaria, filariasis, dengue, Japanese encephalitis and louse-borne typhus.[29] Other kinds of benefits include the maintenance of aesthetic quality, suppression of nuisance causing pests, and the protection of other organisms including endangered species from pests. Pesticides are also used in houses and buildings to protect from structural damage causes by organisms like termites and wood boring insects. The transport sector makes extensive use of pesticides, predominantly herbicides. Also, the herbicides and insecticides are used in maintaining of turf on sports pitches, grounds, golf courses etc.

Due to excessive usage of pesticides, its worldwide consumption is estimated to be approximately 2 million tonnes per year. Out of which 45% is consumed by Europe alone, 25% by USA, and remaining 25% is used by the rest of the world.[29] The world's largest pesticide consumer is Japan and the largest pesticide market is in Asia.[30] In India, the usage of pesticide started in 1948 with the application of DDT. Now, India is the fourth largest global producer of pesticides after the USA, Japan, and China[31,32] In India, the consumption pattern of pesticides is tilted more towards the use of insecticides that too organophosphates in comparison to other pesticides.[7,31] This consumption pattern is sharply different to the rest of the world where herbicide and fungicide are used in higher

proportions.[29] However, the consumption of pesticides in India is only 0.6 kg/ha which is lowest in the world, while in UK and China, it is 5–7 kg/ha and 13 kg/ha, respectively.[32] In India, only 84 out of 230 registered pesticides, are actually used in the agriculture sector, and only 25–30% of the total cultivated area of the nation, i.e. 143 million hectare is under pesticide cover. Consumption pattern of pesticides are highly inconsistent and vary from one region to another. Countries like China, United States, France, Brazil and Japan are the largest pesticide producers, consumers or traders in the world.[30] According to one of the estimates certain countries such as Mauritius, New Zealand, Malaysia, Ireland, Kuwait, Netherlands, Israel, and Chile consumed more than 800 kg fertilizers per hectare of harvested land in 2010.[33] In addition to pesticides some countries such as Mexico, United States and Canada are also using biopesticides. Consumption of biopesticides in these countries accounts for 44% of the world.[30] The use of biopesticides helps in reducing pollution and harmful effects caused due to excessive use of pesticides. In India, with the start of low cost natural and organic farming practices, government is focusing on meagre use of pesticides. States like Sikkim and Himachal Pradesh are front runners in phasing out usage of pesticides for farming practices.

Routes of human exposure to pesticides and their impact on One Health

The environment is considered as a major source of exposure to pesticides. It is estimated that about 47% of the chemical products which are being used are deposited at or adjacent to soil and water resources.[34] Pesticides are found at detectable levels in many compartments of the environment. Once pesticides gets released into environment, they persist there in the form of residues and poses a great risk to live stock and human health. They gets entry into the body and tend to build up in the fatty tissues of living organisms, causing serious harm to the health and a potential loss of biodiversity. Exposure to pesticides can occur directly from occupational, agricultural, and household use, while they can also be transferred indirectly through diet. Farmers and their families are at greater risk to exposure of pesticides than the general population. About 56.7% of the population in India is engaged in agricultural activities and is exposed to the pesticides.[32] The main sources of exposure to pesticides in the population are plant foods (fruits, vegetables, grains) or animal (beef, pork, fish, dairy products, eggs, etc.), and to a lesser extent water, air, soil etc. Pesticide exposure during pregnancy is associated with an increased risk of spontaneous abortion, fetal death and early childhood cancers such as acute lymphocytic leukemia.[35] Pesticides can enter human and animal food chain through various pathways and the portal of entry can be oral, cutaneous, respiratory or ocular.

Effects of pesticides on One Health

Effect on environment: Most of the pesticides are not easily degraded in the environment, i.e. they usually persist for a very long time, such pesticides are considered as persistent organic pollutants (POPs) e.g. aldrin, chlordane, DDT, dieldrin, endrin, heptachlor and hexachlorobenzene.[29,36] This ability to resist degradation and remain in the environment for many years dependon their physical and chemical properties. These POPs have the ability to bioaccumulate and biomagnify, they can be bio-concentrated by up to 70,000 folds relative to their initial concentration.[36] Pesticides pollute soil, leach to underground and surface water and affect other vegetations. In addition to destroying insects or weeds, pesticides can be toxic to other non-target organisms including birds, beneficial insects and other wild flora and fauna. Water contamination by pesticides is widespread. During a survey in India, 58% of drinking water samples drawn from various hand pumps and wells were found to be contaminated.[37] The study conducted on water samples collected from different sites upstream and downstream sections of river Yamuna in Delhi showed that the concentration of aldrin and dieldrin residues ranged from 0.0005–0.05 microgram/ml (upstream) and 0.0001 to 0.1 microgram/ml (downstream), respectively.[38] Surface water samples taken from five lakes of Nainital (North West India) contained 6.054–31.336 µg/l of DDT and 3.121–8.656 µg/l of HCH, in places where insecticide has never been used for vector control.[39] Organochlorine pesticides were detected even in the snow on Nanjiabawa Peak in Tibet, with an elevation of 4,250 m.[30] Once water bodies are polluted with toxic chemicals, it may take years for the contamination to disintegrate or be cleaned up. Also, the increasing incidences of pesticide residues in the dairy products, meat, eggs etc are of a great concern for ensuring food safety and human health. Persistence of chemical fertilizers in the environment resulted in series of undesirable effects through contamination of air, food and water resources. Discharge of industrial effluents, untreated domestic waste and ever increasing use of agrochemicals in modern farming system resulted in contamination of soil

and ground water sources. Most of the pesticide applied cause harm to other non-target organisms; including birds, useful insects, fishes etc. It may take many years to remove the toxic chemicals from water bodies' once contaminated. The heavy and repeated long term treatment of soil with pesticides and chemical fertilizers cause reduction in the populations of beneficial soil microorganisms. Mycorrhizal fungi grow with the roots of many plants and aid in nutrient uptake adversely affected by application of herbicides in the soil.[28] Excessive pesticide use resulted in environmental damage by contaminating soil, water, plants etc. and also increased resistance in the target pest organisms.

Effect on human and animal health: The main sources of pesticides in the human food chain are the foods of animal origin and environmental exposure. It has been concluded that humans are exposed to these toxic compounds via diet in a much higher degree compared to other exposure routes such as inhalation and dermal exposure. The main pathway for the contamination of animal origin food is the ingestion of the contaminated feed and/or water by the animals[40] (Ledoux, 2011). Exposure to pesticides causes a wide range of health problems. Its poses significant risks to the environment ranging from beneficial soil microorganisms, to insects, plants, animals, humans, birds etc. On an average 10,000 deaths occur annually due to use of chemical pesticide worldwide, with 3/4thof these occurring in developing countries.[41] In India, the first report of pesticide poisoning was documented from Kerala in 1958, where more than 100 people died after consuming wheat flour contaminated with parathion.[37] An accidental emission of methyl isocyanate from a pesticide factory located in Bhopal resulted in more than 5,000 deaths. Pesticide impacts on human health are highly variable. In Saran district, Bihar a tragedy happened in 2013 in which more than 30 children died because of consuming of monocrotophos, a deadly organophosphorus pesticide.[31] In a study it is found that pesticide residues in food can prevent the availability of folic acid leading to the birth of children with NTD (Neural Tube Defect).[41] Some pesticides are known to act as endocrine disruptors which elicit theiradverse effects by antagonizing natural hormones in the body. The long-term, low-dose exposure of such chemicals is increasingly linked to human health effects such as immune suppression, hormone disruption, diminished intelligence, reproductive abnormalities, cancer etc.[28] Some of the known endocrine disrupting chemicals which are present in large quantities in our environment include DDT, lindane, atrazine, carbaryl, parathion etc.[42] Pesticides may also induce oxidative stress in body and the stress markers present in plasma linked with POPs exposure leading to generation of free radicals and resulting in many debilitating chronic diseases.[43] Exposure to DDT and its metabolites cause's eggshell thinning as a result of this bald eagle population in the United States declined. OPs were expected to degrade rapidly in the aquatic ecosystem, but researches have shown that they persist days/ weeks and are accumulated by crustacean and fish causing adverse effects. OCs also greatly affects the top predators in terrestrial food chains and accumulates in adipose tissues of animals and humans, transferred to young ones through milk and act as endocrine disruptor.[5] Some herbicides may produce acute toxicity and sublethal effects on fish that reduces their chances for survival.[28] Glyphosate or glyphosate-containing products can cause sublethal effects in fish such as erratic swimming and labored breathing.[44] 2, 4-D herbicides caused physiological stress responses in sockeye salmon and reduced the abilities of food-gathering in rainbow trout.[28] On the basis of effects produced, the impact of pesticides on human health can also be categorized into acute and chronic effects. Acute health effects are sometimes misdiagnosed or not properly recognized as being associated with pesticide toxicity. They often include headache, stinging of the eyes and skin, irritation of the nose and throat, skin itching, appearance of the rash and blisters on the skin, dizziness, diarrhoea, abdominal pain, nausea and vomiting, blurred vision, blindness and very rarely death. Chronic or long-term effects of pesticides may take few hours or as long as months or years to manifest and are usually fatal. They may lead to adverse effects such as a fourfold increased risk of early-onset of Parkinson's disease, shortened attention span, memory disorders, reduced coordination, depression, cancer, reproductive problems including miscarriages, reduced infant development, birth defects etc.[37] Also, long-term pesticide exposure damages the immune system and can cause hypersensitivity, asthma and allergies.

Regulation of Pesticides

Continuous and increased use of pesticides poses a significant risk to environment, animal and human health. Therefore, pesticides must be regulated in order to ensure their safety, marketing, proper use and disposal. They should be properly monitored to avoid unacceptable risks to humans, animals, or the environment. Recognizing proper regulatory standards and management practices of using

the pesticides are the alternative ways to prevent the adverse effect of pesticide residue on the environment. Globally, pesticide regulation was given little attention until the 1940s. In 1962, the publication of Silent Spring highlighted the issues regarding the environmental damage caused by synthetic pesticides. By 1980s, the use of DDT was banned in developed nations, and the need for new and improved pesticide legislation was recognized. In the late 1960s, a new arena was opened in which "integrated pest management" (IPM) was introduced.[3] IPM is a method in which biological predators or parasites are used for controlling the pests. In 1970–1980s, pyrethroids, sulfonylureas, synthetic fungicides triadimefron and metaxyl were introduced. 179 nations including India signed an international treaty in 2001 (Stockholm Convention) that was intended to completely ban twelve Persistent Organic Pollutants (POP's) including DDT.[3] The Stockholm Convention has classified most of the organochlorines as environmental hazards because of persistence and bioaccumulation effect and banned their use [45]. The convention is designed to protect human health and the environment from POPs which have become a leading global issue. In addition to this, signatories are also required to take necessary measures to prevent/control the release of POPs and ensure the safe disposal of such substances when they become waste.

Various countries across the globe usesmaximum residue limits (MRLs) to regulate pesticides. MRLs are defined as the upper legal levels of a concentration for pesticide residues (expressed in mg/kg) in or on food or feed based on good agricultural practices and to ensure the lowest possible consumer exposure. As an example, the European Union has established maximum contents for these compounds in animal feed which can be as low as 5 µg Kg^{-1} for some OCPs in fish feed and β-HCH in cattle feed. In the rest of feed materials these values can be as low as 10 µg Kg^{-1} relative to feedstuff with moisture content of 12%. In India, Ministry of Health and Family Welfare regulates tolerance limits of pesticide and agrochemical in food products through the Food Safety and Standards Act (FSSA), 2006. The Ministry of Agriculture regulates the manufacture, sale, import, export and use of pesticides through the Insecticides Act, 1968 and the Insecticides Rules, 1971. All insecticides (including fungicides and herbicides per Section 3e) are listed in the "Schedule," and must undergo a registration process with the Central Insecticides Board & Registration Committee (CIB & RC). As of May 15, 2019, there are 288 registered insecticides under Section 9(3) of the Insecticides Act, 1968. Although FSSAI vide Food Safety and Standards (Contaminants, Toxins and Residues) Regulations, 2011 has established tolerance limits for various food commodities such as milk, meat, egg, fish, water, food grains, pulses, vegetables, fruits etc., but still such limits for animal feed materials have not yet been set in India.However, the MRLs of a pesticide in feed can be derived on the basis of its legal permissible limit in milk and its rate of transference from feed to milk. In spite of ban, DDT and BHC are still in production in India and are being detected in many matrices. Out of their total production in world India accounts for manufacturing of 77% DDT and 95% BHC.[46]

Conclusion

Pesticides are considered as an effective, quick and inexpensive way of controlling weeds and insect pests. They have proved to be a useful for the farmers as well as for people all around the world through their contributions to increase agricultural yield and by providing benefits to society. Ideally, pesticides should be selective in nature and must not harm other non-target organisms. However, it is difficult to achieve such absolute selectivity and the issue of hazards posed by pesticides to human health and the environment has raised concerns about the safety of pesticides. Therefore the use of pesticides should be properly monitored and regulated in order to ensure the safety of humans, animals and environment. Animal feed plays an important part in the food chain and has implication for the composition and quality of the livestock products that people consume. Under this scenario, it is important to understand the crucial issues like; what are the environmental and health costs of pesticide use. Therefore new methods should be found out in order to minimize the use of harmful pesticides and to maximize the production and ways out of crop protection from insect pests.

References

1. Khush GS. Green revolution: the way forward. Nature Reviews Genetics 2001;2:815.
2. Bourguet D, Guillemaud T. The hidden and external costs of pesticide use. Sustainable Agriculture Reviews 2016;35:120.
3. Mahmood I, Imadi SR, Shazadi K, et al. Effects of pesticides on environment. Plant, Soil and Microbes 2016.pp.253–69.

4. Jawale CA, Rajput KH, Ugale BJ. Assessing the impact of pesticides: An overview. International Journal of Life Sciences 2017;5:474–79.
5. Carvalho FP. Pesticides, environment, and food safety. Food and Energy Security 2017;6:48–60.
6. Devi PI, Thomas J, Raju RK. Pesticide Consumption in India: A Spatiotemporal Analysis. Agricultural Economics Research Review 2017;30(1):163–72.
7. Kumar A, Gill PS, Bedi JS. Multiresidue determination of pesticides in market honey from northern India using QuEChERS approach and assessment of potential risks to consumers. Current Science 2018;115(2):283–91.
8. Kaphalia BS, Seth TD. Organochlorine pesticide contamination in some species of fodder grasses. Environmental Pollution Series B, Chemical and Physical 1982;3(3):231–37.
9. Prameela Devi V, Nanda NV, Hariharan NV. Survey of pesticide residue analysis in water resources and paddy straw samples from some farms of Nellore districts of Andhra Pradesh. Pollution Research 1982;1:21–23.
10. Chawla RP, Kalra RL. Harvest time residues of DDT and HCH in Punjab wheat crop untreated with these chemicals. Agro-ecosystems 1983;8:255–57.
11. Singh P P, Battu RS, Kalra RL. Insecticide residues in wheat grains and straw arising from their storage in premise treated with BHC and DDT under malaria control program. Bulletin of Environmental Contamination and Toxicology 1988;40:696–02.
12. Dikshith TSS, Kumar SN, Raizada RB, et al. Organochlorine insecticide residues in cattle feed. Bulletin of Environmental Contamination and Toxicology 1989;43(5):691–96.
13. Mukherjee I, Gopal M. Insecticide residues in baby food, animal feed, and vegetables by gas liquid chromatography. Bull Environ Contam Toxicol 1996;56:381.
14. Kang BK, Singh B, Chahal KK, Battu RS. Contamination of feed concentrate and green fodder with pesticide residues. Pestic Res J 2002;4:308–12.
15. Babu GS, Farooq RS, Ray PC, et al. DDT and HCH residues in basmati rice (oryza sativa) cultivated in Dehradun (India). Water, Air, and Soil Pollution 2003;144:149–57.
16. Deka SC, Barman N, Baruah AALH. Monitoring of pesticide residues in feed, fodder and butter in Assam. Pestic Res J 2004;16:86–89.
17. Aulakh RS, Gill JPS, Bedi JS, et al. Organochlorine pesticide residues in poultry feed, chicken muscle and eggs at a poultry farm in Punjab, India. J Sci Food Agric 2005;86:741–44.
18. Sharma V, Wadhawa BK, Stan HJ. Multiresidue analysis of pesticides in animal feed concentrate. Bulletin of Environmental Contamination and Toxicology. 2005;74:342–49.
19. Nag SK, Raikwar MK. Persistent organochlorine pesticide residues in animal feed Environ Monit Assess. 2011;174:327–35.
20. Sharma HR, Kaushik A, Kaushik CP. Organochlorine pesticide residues in fodder from rural areas of Haryana, India, Toxicological & Environmental Chemistry. 2013;95(1):69–81.
21. Reddy MVB, Reddy YR. Pesticide residues in animal feed and effects on animals and its products with special reference to endosulfan. International Journal of Research in Ayurveda and Pharmacy. 2015;6(3):371–74.
22. Kaur H, Aulakh RS, Bedi JS, et al. Occurrence of pesticide residues in fodder from Punjab (India): temporal and spatial variation. Indian Journal of Animal Sciences 2015;85(7):764–766.
23. Karabasanavar N, Singh SP, Ahmad AH, et al. Monitoring of Animal Feed and Fodder Samples for Endosulphan Residues. Journal of Veterinary Public Health 2015;13(2):119–22.
24. Khilare RS, Khurana R, Narang G, et al. Occurrence of some organochlorine pesticide residues in poultry feed and meat. Haryana Veterinarian 2016;55(2):120–24.
25. Korrapati K, Kotha K, Nelapati K. Determination of organophosphoruspesticide residues in fodder samples along Musi River belt, Hyderabad, India. Int J Curr Microbiol App Sci 2018;7(4):2535–45.
26. Sharifzadeh MS, Abdollahzadeh G, Damalas CA, et al. Farmers' criteria for pesticide selection and use in the pest control process. Agriculture 2018;8:24.
27. Choudhary S, Yamini NR, Yadav SK, et al. A review: Pesticide residue: Cause of many animal health problems. Journal of Entomology and Zoology Studies 2018;6(3):330–33.
28. Aktar MW, Sengupta D, Chowdhury A. Impact of pesticides use in agriculture: their benefits and hazards. Interdiscip Toxicol 2009;2(1):1–12.
29. Yadav IC, Devi NL, Syed JH, et al. Current status of persistent organic pesticides residues in air, water, and soil, and their possible effect on neighboring countries: a comprehensive review of India. Science of the Total Environment 2015;511:123–37.
30. Zhang W, Jiang F, Ou J. Global pesticide consumption and pollution: with China as a focus. Proceedings of the International Academy of Ecology and Environmental Sciences 2011;1:125.
31. Bhardwaj T, Sharma JP. Impact of pesticides application in agricultural industry: An Indian scenario. International Journal of Agriculture and Food Science Technology 2013;4:817–22.
32. Koli P, Bhardwaj NR. Status and use of pesticides in forage crops in India. Journal of Pesticide Science 2018;18:4.
33. Liu Y, Pan X, Li J. A 1961–2010 record of fertilizer use, pesticide application and cereal yields: a

review. Agronomy for sustainable development 2015;35:83–93.

34. Garcia FP, Ascencio SYC, Oyarzun JCG, et al. Pesticides: classification, uses and toxicity. Measures of exposure and genotoxic risks. Journal of Research in Environmental Science and Toxicology 2012;1:279–93.
35. Sanborn MD, Cole D, Abelsohn A, et al. Identifying and managing adverse environmental health effects: Pesticides. Canadian Medical Association Journal 2002;166:1431–36.
36. Kim KH, Kabir E, Jahan SA. Exposure to pesticides and the associated human health effects. Science of the Total Environment 2017;575:525–35.
37. Kumar S, Sharma AK, Rawat SS, J et al. Use of pesticides in agriculture and livestock animals and its impact on environment of India. Asian Journal of Environmental Science 2013;1:51–57.
38. Nair A, Dureja P, Pillai MKK. Levels of aldrin and dieldrin in environmental samples from Delhi. Science of the Total Environment 1991;108:255–59.
39. Dua V, Kumari R, Johri R, et al. Organochlorine Insecticide Residues in Water from Five Lakes of Nainital (U. P.), India. Bull Environ ContamToxicol 1998;60:209.
40. Ledoux, M. Analytical methods applied to the determination of pesticide residues in foods of animal origin: A review of the past two decades. J Chromatogr A. 2011 Feb 25;1218(8):1021-36.
41. Gore MM, Aryan PK. Pesticides use and their impact on environment and health in Uttar Pradesh: A review. Environment Conservation Journal 2015 16:117–27.
42. Sarwar M. The dangers of pesticides associated with public health and preventing of the risks. International Journal of Bioinformatics and Biomedical Engineering 2015;1:130–36.
43. Abdollahi M, Ranjbar A, Shadnia S, et al. Pesticides and oxidative stress: A review. Medical Science Monitor 2004;10:141–47.
44. Liong PC, Hamzah WP, Murugan V. Toxicity of some pesticides towards fresh water fishes. Malays Agric J 1988;54(3):147–156.
45. Jayaraj R, Megha P, Sreedev P. Organochlorine pesticides, their toxic effects on living organisms and their fate in the environment. InterdiscipToxicol 2016;9(3-4):90–100.
46. Chauhan RS, Singhal L. Harmful effects of pesticides and their control through cowpathy. International Journal of Cow Science 2006;2:61–70.

Review Article

Journal of Animal Feed Science and Technology
Volume 7 Number 2, July – December 2019
DOI: http://dx.doi.org/10.21088/jafst.2321.1628.7219.4

Potential Use of Fat Replacers for Development of Functional Food of Animal Origin

Meena Goswami[1], Tanuja[2], Vikas Pathak[3], Heena Sharma[4], Sadhana Ojha[5]

[1]Assistant Professor, [2]Phd Scholar, [3]Professor & Head, [5]Phd Scholar, Department of Livestock Products Technology, College of Veterinary Sciences and Animal Husbandry, DUVASU, Mathura, Uttar Pradesh 281001, India. [4]Scientist, Department of Dairy Technology Division, National Dairy Research Institute, Karnal, Haryana 132001, India.

How to cite this article:
Meena Goswami, Tanuja, Vikas Pathak et al. Potential Use of Fat Replacers for Development of Functional Food of Animal Origin. Journal of Animal Feed Science and Technology. 2019;7(2):81–86.

Abstract

Fat replacers are the substances which provide the health benefits to the consumer by reducing the incidence of cardio-vascular diseases. Fat replacers may be either carbohydrates or protein based and used singly or in combination in the products. Today's consumer is very demanding and choosy for their nutritional requirements and mostly preferred the food items having low fat, low salt, fibre rich and antioxidative properties. So this review explained the importance of fat replacers in milk and meat products with their brief classification, present status and role.

Keywords: Fat replacer; Fat mimetics; Fat substitute; Low calorie fat; Meat products; Milk products.

Introduction

Fat is a very important ingredient contributing to the texture, flavor and overall perception of cookies. Fat shortens a dough by weakening its gluten network, resulting in the baked product being softer, breaking easily and having a more tender mouth feel. Fat can trap air during beating and mixing, producing a batter that consists of masses of tiny air bubbles trapped within droplets of fat (Zoulias et al., 2002). Fat also imparts richness and tenderness, improving flavor and mouth feel to processed meat products (Pareyt and Delcour, 2008). However, an excess of energy intake and the consequent high amount of fat (especially saturated fat) is associated with health disorders such as obesity, cancer, high blood cholesterol and coronary heart disease (Akoh, 1998). In fact, the total fat content should not be higher than 30 percent of the daily energy intake.

Corresponding Author: Meena Goswami, Assistant Professor, Department of Livestock Products Technology, College of Veterinary Sciences and Animal Husbandry, DUVASU, Mathura, Uttar Pradesh 281001, India.

E-mail: dr.goswami2008@yahoo.co.in

Received on 28.10.2019; **Accepted on** 28.11.2019

Saturated fats should not be more than 10 percent and monounsaturated and polyunsaturated 20 percent of the total energy intake (USDA, 1999). Red meat like mutton, carabeef and beef contain more saturated fat than that of white meat like rabbit and chicken meat. Many meat based snack products are also now marketed with "fat free" slogans, (97-98% fat-free) and in fact, most have been low in fat since their early development. The flavor intensity, juiciness, and tenderness of meat products are directly correlated with fat content and that reducing fat content reduces overall acceptability (Brewer, 2012). Reducing the fat content of ground beef to 10% often results in a cooked product that is bland and dry with a hard, rubbery texture (Keeton, 1994; Youssef and Barbut, 2010). When the total fat content is reduced, optimizing the remaining fat content and cooking parameters to produce a good quality finished product are necessary to meet consumer expectations and ultimately result in consumption of lower fat, lower calorie products.

Fat replacers are substances of carbohydrate or protein nature which can imitate the functional and sensory properties of fat without increasing caloric intake (Lindsay, 2000). They need to be able to replicate all or some of the functional properties of fat in a fat-modified food. The fat in foods can be lowered by simple techniques such as dilution with water or substituting with ingredients such as fruit purees or with use of compounds developed by food technologists (Ruthig et al., 2001).

Fat replacer are those substances which take place of all or some of the fat in a food and give the food a same texture, taste, juiciness and mouthfeel to the original full- fat food. Fat provides some essential functions in the food like carry the fat soluble vitamins (A, D, E and K), act as a precursor of prostaglandin and it is the source of essential fatty acids linoleic and linolenic acids. Various health organizations have recommended lowering of daily intake of dietary fat to an average of 30% of total calories, consuming less than 300 mg of cholesterol per day and limiting saturated fat to less than 10% of total calories (Matulis et al., 1995).

The AHA (American Heart Association) recommends that those with elevated LDL cholesterol levels or cardiovascular disease restrict saturated fats to <7% of calories. To achieve a more healthful dietary pattern, current dietary guidelines recommend increasing intake of fruits, vegetables, and grains and modifying the type and amount of fat consumed (Krauss et al., 2000; Wylie-Rosett, 2002). The high intake of fat is highly associated with certain diseases like obesity, high blood cholesterol level, cancer and coronary heart disorders. But reduction of fat content leads to a firmer, rubbery, less replaced in food products by traditional techniques such as substituting water (Chronakis, 1997) or air for fat, using lean meats in frozen entrées (Hsu and Sun, 2005), skim milk instead of whole milk (Zalazar et al., 2002) in frozen desserts (Specter and Setser, 1994) and baking instead of frying (Haumann, 1986) for manufacturing or preparing snack foods.

Classification of fat replacer

Basically fat replacers are categorized into three categories:

1. Fat Mimetics
2. Fat Substitutes
3. Low Calorie fat

1. *Fat Mimetics*

Fat mimetics are defined as a partial replacement for fat by mimicking or imitating a particular function, but not all functions of fat in a food. They are commonly carbohydrate and protein based fat replacer.

- Carbohydrate based fat replacer included guar gum, polydextrose (litess), gum Arabic, xanthum gum, carrageenan, modified food starches, cellulose, oat fiber and wheat fiber. They all are non caloric sources.
- Protein based included simplesse, trailblazer, and zein protein.

In those the guar gum is extracted from leguminous seeds and xanthum gum by aerobic fermentation of *Xanthum campestris.* Carrageenan is extracted from red sea weed *Chondrus crispus* (Irish moss).

Simplesse is obtained from white egg protein and milk protein. Trailblazer is from white egg protein & serum protein mixed with xanthan gum.

2. *Fat Substitute*

They are basicly fat based fat replacer which have the properties of fat and oils but are not absorbed or metabolised by the body. They commonly included Olestra, Sorbestrin and Esterified propoxylated glycerol esters (EPGs).

a. *Olestra*

Olestra made from highly unsaturated fatty acids is liquid at room temperature; olestra made from highly saturated fatty acids is solid (Harrigan

and Breene, 1989). Olestra has a zero calorific value because it neither digested nor absorbed by the gastric or pancreatic enzymes. Due to no absorption it is totally excreted out the high cholesterol from the body and plays a main role in reducing the cholesterol level in hypercholestrimic patient. But there are some side effects of olestra in some people like it reduces the absorption of fat soluble vitamins in the body, produces abdominal cramps and loose stools. It is mainly used in the preparation of savory snacks such as potato and corn chips, cheese puffs and crackers and for frying of savory snacks (Akoh, 1998; Cooper et al., 1997; Peters et al., 1997]. Some people eating large amounts of olestra snacks may experience common gastrointestinal tract symptoms such as stomach discomfort or changes in stool consistency, similar to symptoms accompanying other dietary changes but these symptoms present no health risks (Akoh, 1998; Cooper et al., 1997]. As a result, the Food and Drug Administration (FDA) requires that food containing olestra be labeled with the statement: "This Product Contains Olestra".

b. *Sorbestrin*

Sorbestrin is the mixture of tri-, tetra-, and pentaesters of sorbitol and sorbitol anhydrides with fatty acids. The caloric value of sorbestrin is 1.5 kcal/g. It is commonly used in salad dressings, baked goods, and frying (Lucca and Tepper, 1994).

3. *Low Calorie Fat*

They generally included medium chain triglycerides (MCT), caprenin and Salatrim. The caloric value of them is slightly lower than 9 kcal/g.

a. *Caprenin*

Caprenin (caprocaprylobehenic triacylglyceride), (The Procter & Gamble Co.), is manufactured from glycerol by esterification with caprylic (C8:0), capric C10:0), and behenic (C22:0) fatty acids (Costin and Segal, 1999). behenic acid is only partially absorbed and capric and caprylic acids are more readily metabolized than other longer chain fatty acids, caprenin provides only 5 kcal/g (Akoh, 1998; Lucca and Tepper, 1994). Caprenin, in combination with polydextrose, was commercially available briefly in reduced-calorie and reduced- fat chocolate bars (Sandrou and Arvanitoyannis, 2000). Caprenin's functional properties are similar to those of cocoa butter (Lipp and Anklam, 1998). As a result, caprenin is suitable for use in soft candy and confectionery coatings (Lucca and Tepper, 1994).

b. *Salatrim*

Salatrim is a randomized triglyceride containing short and long chain fatty acids. The short chain fatty acids are acetic, propionic and butyric, while long chain fatty acids include stearic acid. The stearic acid and other long chain fatty acids are poorly absorbed. The calorific value of salatrim is about 5 kcal/g. It is commonly used in chocolate-flavored coatings, deposited chips, caramels and toffees, fillings and inclusions for confectionary, peanut spread and dairy products such as sour cream, frozen dairy desserts, and cheese (Lucca and Tepper, 1994; Kosmark, 1996).

Present status of fat replacers

Market for fat replacers will reach 280,100 MT with an annual growth rate of 6.03% predicted between 2011 and 2015. Meat industry is the largest recipients of fat replacers because low calorie and reduced-fat foods such as low-fat meat products are gaining huge popularity in these markets. The carbohydrate and protein based fat replacer continue lead the market of fat replacer that offer dietary and processing benefits to the consumers.

Role of fat replacer in meat and meat products

In case of red and white meat, the red meat contains more than 2.64 times of saturated fatty acids so the risk of certain type of disorders are mainly related with juicy product with dark color and more cost (Trout et al., 1992; Cavestany et al., 1994; Keeton, 1994; Paneras et al., 1996; Desmond et al., 1998; Kirchner et al., 2000). So for reducing the fat, use of fat replacer is a best option to maintain the quality and acceptability of products. The lot of work was done on the use of fat replacer in meat and meat products and in production of low fat meat products.

In the early 1990s, work on acceptable reduced and low-fat sausage systems used added water and carrageenan to low-fat sausages containing 8% fat without deleterious effects on lipid or color stability (Bradford et al., 1993). A year later, Osburn and Keeton (1994) developed acceptable low-fat prerigor pork sausages, containing 10% fat, with 10–20% konjac flour gel. Carrageenan was used in the preparation of low fat frankfurter (Foegeding and Ramsey, 1987) and in pork nuggets (Berry, 1994) as a fat replacer. Carrageenan in pork nuggets increasing cooking yield and in the preparation of meat patties carrageenan reduced the fat as well as calorie content of product. Some workers observed that guar gum increases the emulsion stability by providing thickening and gelling properties.

Barbut and Mittal (1996) found that in cooked low fat sausages, fat content decreased by 52–60% and moisture content decreased by 61-65% by use of carboxymethyl cellulose (CMC) as fat replacer.

Choi et al. (2012) used a Surimi like material (SLM- 20%) as a fat replacer in pork patties, obtained from longissimus dorsi muscle which reduced fat upto 1.76% as well as improving the organoleptic properties, tenderness and overall acceptability. The synthetic fat replacer olestra has also a role in meat industry as a frying medium for preparation of prebrowned meat items.

Berry (1997) used combination of sodium alignate and modified tapioca starch with 7 and 14 percent added water levels and observed increase in juiciness, tenderness and cooking yield of low-fat (<10%) beef patties. The role of sodium alginate as fat replacer was studied by Kumar et al. (2007) in the processing of low fat ground pork patties. Sodium alginate reduces the cholesterol content as well as calories and improved the shelf life of pork patties. Bhat et al. (2017) also reported that well accepted functional chicken cutlets could be prepared by incorporation of 3% mango peel powder without any adverse effect on sensory attributes.

Nisar et al. (2009) evaluated the efficacy of tapioca starch as a fat replacer in low fat buffalo meat patties and concluded that the low-fat buffalo meat patties incorporated with tapioca starch had higher cooking yield, better dimensional parameters and substantially higher scores for sensory attributes in comparison to the other low-fat buffalo meat patties and high-fat control patties. Ground poppy seeds upto 20% level was incorporated in meat burgers as a fat replacer which decreases saturated fatty acids as well as cholesterol content in meat burgers (Gök et al., 2011). Park et al. (2000) also observed the improvement in cooking yield, textural and sensory properties of low-fat pork patties (10%) with the incorporation of various hydrocolloids (Sodium alignate, Carboxy Methyl cellulose and Xanthan gum). The amorphous cellulose gel was used as fat replacer in fermented sausages which replaced the pork back fat and reduced the cholesterol content from sausages (Campagnol et al., 2012). Goswami et al. (2019) also reported significant ($p < 0.05$) increase in cooking yield of carabeef cookies incorporated with different levels of plum pulp powder as fat replacer.

Role of fat replacers in milk and milk products

Roland et al. (1999) examined the effects of individual fat replacers on the physical and sensory properties of fat-free ice cream. Ice creams (≤0.5% milk fat) were formulated with maltodextrin, milk protein concentrate, or polydextrose. The sensory analysis panel scored maltodextrin as best overall as a single fat replacer in fat-free ice cream. These results suggest the need for development of fat replacer blends to optimize quality of fat-free frozen desserts.

Two protein-based fat replacers (Simplesse® D100 and Dairy-Lo®) and two carbohydrate-based fat replacers (Stellar™ 100X and Novagel™ RCN-15) was used in the development of low fat mozzarella cheese. Distribution of the fat replacers within the cheese was influenced by the extent of microparticulation of the fat replacer, size of the fat replacer particles, and processing steps that caused an interaction between the fat replacer and the caseins in milk (McMahon et al., 1996).

The textural, melting and sensory properties of low-fat fresh kashar cheeses (70% fat reduction) produced by using two protein-based fat replacers (1.0% w/w Simplesse® D-100 and 1.0% w/w Dairy-Lo™) and one carbohydrate-based fat replacer (5.0% w/w Raftiline® HP) were examined during the storage period for 90 days. The results indicated that Simplesse® D-100 and Raftiline® HP can improve the texture and sensory properties of low-fat fresh kashar cheese (Koca and Metin, 2004). As per Anita et al. (2018), citrus fruits and their by products could be used as efficient fat replacers for development of different functional dairy as well as meat products.

Ohmes et al. (1998) demonstrated the importance of fat as flavor modifier and the importance of certain fat replacer as aids in improving texture and determined the relative effects of milk fat, nonfat milk solids, or each of three whey protein type fat replacers on the flavor and texture attributes of vanillin-flavored ice cream.

Conclusion

The demand of animal products is increased with a frequent rate and hugely among all age group consumers. The red meat is a good source of iron, zinc, phosphorus and other minerals with richest amount of various vitamins. But the red meat contains 2.64 times more saturated fat than white meat so there is greater risk of certain types of major diseases. Similarly the fat content of certain types of milk products is also very high from health point of view. The fat percent of cheese, ice-cream, whole milk, butter etc. is much higher that is mostly replaced by combination of one or two

or more fat replacers because no single fat replacer provides all the beneficial properties. These fat replacers improved the functional as well as sensory properties of milk and meat products and enhanced the shelf life.

The guidelines recommended that daily dietary fat intake should be less than 30% of calories and saturated fats to <10% of total energy intake. So for these dietary goals consumer are selected the food containing low fat like lean meat, poultry and fish, low fat or non fat dairy products, dressings, steamed products etc. for regulating the body cholesterol and fat. The diet is modified with the incorporation of fat replacers and certain functional ingredients for reducing the prevalence of various major diseases. Fat replacers are improved the sensory attributes like texture, flavor, aroma, color and appearance, mouth coating etc. as well as the perishability and functionality of products are also enhanced. Due to presence of so many valuable qualities in fat replacers, the market value for these fat replacers and low fat milk and meat products increase with a very fast rate among the younger generation.

References

1. Akoh CC. Fat replacers. Food Technology, 1988; 52(3):47–53.
2. Anita C, Pathak V, Goswami M, et al. Efficiency of citrus fruist to improve functional properties of livestock products. Journal of Animal Feed Science and Technology, 2018;6:59–66.
3. Barbut S and Mittal GS. Effect of three cellulose gums on the texture profile and sensory properties of low-fat frankfurters. International Journal of Food Science and Technology, 1996;31:241–47.
4. Berry BW. Sodium alginate plus modified tapoica starch improves properties of low-fat beef patties. Journal of Food Science, 1997;62(6):1245–49.
5. Berry BW. Fat level and freezing temperature affect sensory, shear, cooking and compositional properties of ground beef patties. J. Food Sci. 1993; 58:34-37.
6. Bhat A, Kumar A, Sheikh S, et al. Utilisation of Mango Peel Powder as Phytopreservative in the Refrigeration Storage of Chicken Cutlets International Journal of Livestock Research, 2017;7(1):90–99.
7. Bradford DD, Huffman DL, Egbert WR. et al. Potassium lactate effects on low-fat fresh pork sausage chubs duringsimulate d retail distribution. Journal of Food Science, 1993;58(6):1245–48.
8. Brewer MS. Reducing the fat content in ground beef without sacrificing quality: A Review. Meat Science, 2012;91:385–95.
9. Campagnol PC, Dos Santos BA, Wagner R, et al. and Pollonio MA. Amorphous cellulose gel as a fat substitute in fermented sausages. Meat Science, 2012;90(1):36–42.
10. Cavestany M, Jimenez-Colmenero F, Solas MTR. and Carballo, J. Meat Science, 2012;34:27–37.
11. Choi YM, Choe JH, Cho DK and Kim BC. Practical use of surimi like material made from Porcine longissimus dorsi muscle for the production of low-fat pork patties. Meat Science, 2012;90(2):292–96.
12. Chronakis IS. Structural-Functional and Water-holding Studies of Biopolymers in Low Fat Content Spreads. Lebensmittel Wis-s-u-Technology, 1997;30:36–44.
13. Cooper DA., Webb DR and Peters JC. Evaluation of the potential for olestra to affect the availability of dietary phytochemicals. Journal of nutrition, 1997;127:1699S–1709S.
14. Costin GM. and Segal R. Alimente funcționale, Ed. Academica, Galați, 1999;188–02.
15. Desmond EM, Trout DJ and Buckley DJ. Lebensmittel Wis-s-u-Technolog. 1998;31:653–57.
16. Foegeding EA and Ramsey SR. Rheological and water holding properties of gelled meat batters containing iota-carrageenan, kappa-carrageenan or xanthan gum. Journal of Food Science,1987;52(3):549–53.
17. Gök V, Akkaya L, Obuz E and Bulut S. Effect of ground poppy seed as fat replacers on meat burgers. Meat Science, 2011;89(4):400–12.
18. Goswami M, Sharma BD, Mendiratta SK, et al. Efficiency of dried plum powder as fat replacer in buffalo meat incorporated cookies. Fleischwirtschaft international, 2019;3:53–57.
19. Harrigan KA and Breene WM. Fat substitutes: Sucrose esters and simplesse. Cereal Foods World, 1989;34:261–67.
20. Haumann BF. Getting the fat out – researchers seek substitutes for full-fat fat. Journal of the American Oil Chemists' Society, 1986;63:278–88.
21. Hsu SY and Sun LY. Comparisons on 10 non meat protein fat substitutes for low-fat Kung-wans, Journal of Food Engineering, 2005;69:32–46.
22. Keeton JT. Low-fat meat products-Technological problems with processing. Meat Science, 1994;36:261–76.
23. Kirchner MJ, Beasleyi LC, Harris KB. et al. Journal of Food Composition & Analysis, 2000;3:253–54.
24. Koca N and Metin M. Textural, melting and sensory properties of low-fat fresh kashar cheeses produced by using fat replacers. International Dairy Journal, 2004;14(4):365–73.
25. Kosmark R. Salatrim: Properties and applications, Food Technology, 1996;50:98–101.

26. Krauss RM, Eckel RH and Howard B, et al. American Heart Association's Dietary Guidelines: revision 2000: A statement for healthcare professionals from the Nutrition Committee of the American Heart Association. Circulation, 2000;102:2284–299.

27. Kumar M, Sharma BD and Kumar RR. Evaluation of sodium alignate as a fat replacer on processing and shelf-life of low-fat ground pork patties. Asian-Australasian. Journal of Animal Science, 2007;20(4):588–97.

28. Lindsay RD. Aditivos alimentarios. In:Owen R. Fennema (Ed.), Química de los alimentos edn. 2a. Acribia, 2000; Pp 949.

29. Lipp M and Anklam E. Review of cocoa butter and alternative fats for use in chocolate – Part A. Compositional Data, Food Chemistry, 1998;62:73–97.

30. Lucca PA and Tepper BJ. Fat replacer and the functionality of fat in foods. Trends in Food Science and Technology, 1994;5:12.

31. Matulis RJ, McKeith FK, Sutherland JW. and Brewer, M. S. Sensory characteristics of frankfurters as affected by salt, fat, soy protein, and carrageenan. Journal of Food Science, 1995;60:48–54.

32. McMahon DJ, Alleyne MC, Fife RL and Oberg, CJ. Use of Fat Replacers in Low Fat Mozzarella Cheese. Journal of Dairy Science, 1996;79(11):1911–921.

33. Nisar PU, Chatli MK and Sharma DK. Efficacy of tapioca starch as a fat replacer in low-fat buffalo meat patties. Buffalo Bulletein, 2009;28(1):18–25.

34. Ohmes RL, Marshall RT. and Heymann H. Sensory and Physical Properties of Ice Creams Containing Milk Fat or Fat Replacers. Journal of Dairy Science, 1998;81(5):1222–228.

35. Osburn WN and Keeton JT. Konjac Flour Gel as Fat Substitute in Low-fat Prerigor Fresh Pork Sausage. Journal of Food Science, 1994;59:484–89.

36. Paneras EO, Boulkas JG and Papadina SN. Lebensmittel Wis-s-u-Technolog, 1996;29:507–14.

37. Pareyt BY and Delcour AJ. The role of wheat flour constituents, sugar, and fat in low moisture cereal based products: a review on sugar-snap cookies. Critical Reviews in Food Science and Nutrition, 2008;48(9):824–39.

38. Park CK, Song HIK, Nam JH, et al. Effect of hydrocolloids on physico-chemical, textural and sensory properties of pork patties Journal of the Korean Society of Food Science and Nutrition, 2000;29(4):586–91.

39. Peters JC and Lawson KD, et al. Assessment of the Nutritional Effects of Olestra, a Nonabsorbed Fat Replacement: Introduction and Overview. Journal of Nutition. 1997;127:1539–46.

40. Roland AM, Lance G, Phillips and Boor KJ. Effects of Fat Replacers on the Sensory Properties, Color, Melting, and Hardness of Ice Cream. Journal of Dairy Science, 1999;82(10):2094–100.

41. Ruthig DJ, Sider DM and Gill KA. Health benefits of dietary fat reduction by a novel fat replacer: Mimix. International Journal of Food Science and Nutrition, 2001;52:61–69.

42. Sandrou DK and Arvanitoyannis IS. Low fat/ Calorie foods: Current state and prospective. Critical reviews in Food Science and Nutrition, 2000;40(5):427–47.

43. Specter SE and Setser CS. Sensory and Physical Properties of a Reduced Calorie Frozen Dessert System Made with Milk Fat and Sucrose Substitutes. Journal of Dairy Science, 1994;77:708–17.

44. Trout ES, Hunt MC. and Johnson DE. Journal of Food Science, 1992;57:19.

45. USDA Nutrient Database for standard References–Release United State Department of Agriculture, Washington, DC. 1999.pp.132.

46. Wylie-Rosett J. Fat substitutes and health. An Advisory From the Nutrition Committee of the American Heart Association. 2002.

47. Youssef MK and Barbut S. Physiochemical effects of the nature of the lipid phase and protein level on meat emulsion stability, texture and microstructure. Journal of Food Science, 2010;75(2):108–114.

48. Zalazar CA, et al. Effect of moisture level and fat replacer on physicochemical, rheological and sensory properties of low fat soft cheeses. International Dairy Journal, 2002;12:45–50.

49. Zoulias EI, Oreopoulou V and Tzia C. Textural properties of low-fat cookies containing carbohydrate-or protein-based fat replacers. Journal of Food Engineering, 2002;55:337–42.

Review Article

Journal of Animal Feed Science and Technology
Volume 7 Number 2, July – December 2019
DOI: http://dx.doi.org/10.21088/jafst.2321.1628.7219.5

Mycotic Abortions in Bovine: A Review

D Rani Prameela

Professor & Head, Department of State Level Diagnostic Laboratory, Sri Venkateswara Veterinary University, Tirupati, Andhra Pradesh 517502, India.

How to cite this article:
D Rani Prameela. Mycotic Abortions in Bovine: A Review. Journal of Animal Feed Science and Technology. 2019;7(2):87–91.

Abstract

Mycotic abortion is an important reproductive problem of cattle all over the world. It is caused by a number of different species of fungi and yeasts. These usually occur during the winter and spring months, since this is when cows are often kept in total confinement and can be exposed to moldy hay or silage. The epizootiology of the disease is not clearly understood but it is assumed that mouldy hay, straw and feeding stuffs are the most probable transmitting agents. The mold spores are thought to reach the placenta and fetus through the blood supply of the cow, although the way that they gain access to the circulatory system is not well understood. Mycotic abortion in cattle have been recorded usually after first 6 months of gestation. Aborted animals usually suffer from retention of placenta. Fungal abortions tend to occur sporadically although on some occasions a significant percentage (10–20%) of the pregnant animals in a herd may be affected. No treatment has yet been evolved for such abortions.

Keywords: Mycotic abortion; Bovine; Epizootiology; Fungal abortions.

Introduction

Abortion in cattle is a serious problem everywhere in the world where these animals are reared. The implication of fungi in abortion in cattle has received increasing attention during recent years and it is now recognized that mycotic infection contributes significantly to the annual losses from abortion.

Mycotic abortion can cause great economic loss to the stockholders and also a loss of animal proteins to human population which is already facing a very serious problem of shortage of animal proteins. The first record of a fungus isolated from bovine foetal membranes was of *Mucor rhizopodiformis (Rhizopus cohnii)* found growing in a gravid uterus by Theobald Smith (1920).

Mycotic abortion, also known as fungal abortion or mycotic placentitis, is caused by different species of fungi and yeasts. About 35 different species of fungi have been known to cause abortion, *Aspergillus fumigatus* being the most commonly diagnosed casual organism (Jenson et al., 1993).

Corresponding Author: D Rani Prameela, Professor & Head, Department of State Level Diagnostic Laboratory, Sri Venkateswara Veterinary University, Tirupati, Andhra Pradesh 517502, India.

E-mail: raniprameela.dr@gmail.com

Received on 04.06.2019; **Accepted on** 23.10.2019

This form of abortion occurs sporadically and its prevalence may be influenced by poor quality contaminated fodder harvested in wet seasons. *Aspergillus fumigatus* can proliferate in damp hay, in poor quality silage and in brewers grains. Infection, which reaches the uterus haematogenously, causes placentitis leading to abortion late in gestation. Affected cows usually show no signs of systemic illness. Intercotyledonary areas of the placenta are thickened and leathery and the cotyledons are necrotic. Aborted foetuses may have raised cutaneous plaques, resembling ringworm lesions.

The prevalence of mycotic abortion in cattle is influenced by climatic and other environmental factors. Reports from some regions suggest that fungi may be involved in 7% of bovine abortions (Knudtson and Kirkbride, 1992). Although Aspergillus species account for the majority of cases in many countries, *M. Wolfi, Absidia species, Mucor species and Rhizopus species have also been implicated* and, in some regions, may predominate. Abortion which occurs late in gestation, is often linked to the feeding of mouldy hay or silage. The location of lesions on cotyledons suggest haematogenous infection of the uterus, possibly from a pulmonary or enteric source. The cotyledons are enlarged and necrotic, and the intercotyledonary placental tissue is thickened and leathery. Vasculitis, associated with hyphal invasion, is demonstrable in sections of affcetd cotyledons. Occasionally lesions may be observed grossly on the skin of aborted foetuses.

Abortion due to *M. Wolfi,* an important cause of mycotic abortion may be followed within days by an acute fibrinonecrotic fungal pneumonia (Carter et al., 1973). Because of the difficulty in isolating *M. Wolfi* from autolysed tissues, abortion caused by this organism may be under- diagnosed (MacDonald and Corbel, 1981).

Previous reports indicated that > 60% of cases are caused by uncomplicated infection with *Aspergillus fumigatus;* zygomycetes *(Absidia, Mortierella, Rhizomucor, Rhizopus)* accounted for about 20% of cases, and the remaining 20% were caused by a wide range of opportunistic filamentous fungi and yeasts.

Some of possible pathogens of mycotic abortion include:

Mucor rhizopodiformis,

Absidia corymbifera, Absidia ramosa,

A. flavus, A. fumigates, A. nidulans, A. terreus, A. niger, A. versicolnr

Rhizopus pusillus, Rhizomucor pusillus

Rhizopus arrhizus, Rhizopus boyinus,

Kontospora lanuginose,

Mortierella polycephala,

Polystictus versicolor,

Mucordisperses

Mortierella zychae, Mortirella wolfii,

Candida tropiculis,

Nicardia asterodies, Mortirellla wolfii.

Mycotic Placentitis

The chief fungus associated with mycotic abortion is *Aspergillus fumigatus*, which has been recorded from over 60% of cases. *Absidia ramosa* and *Absidia corymbifera* are also frequent isolates, but the remaining species are rarely reported. Fungi have been recovered from the placenta, amniotic fluid, foetal stomach contents and skin lesions. Very rarely have isolations been made from other organs of aborted foetus (Figs. 1 and 2).

Fig. 1: Mycotic abortion—Portion of bovine placenta with thickened cotyledons infected with Aspergillus fumigatus.

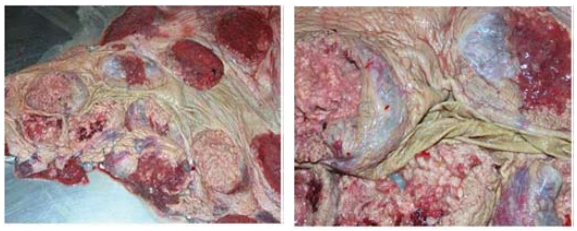

Fig. 2: Mycotic Placentitis.

Epizootiology

Geographical distribution and transmission

The disease has been reported from Europe, North America, South Africa, Australia and parts of Asia. The exact mode of transmission of mycotic abortion is not fully understood and route and source of infection remain unknown. There are

indications that infection is originally derived from the spores of fungi present in large number in the mouldy hay, straw and feeding stuffs and hence in the air of cowsheds (Ainsworth and Austwick, 1959). However, there is no evidence of animal to animal transmission of the disease. The disease is sporadic and rarely affects more than 1 or 2 animals in a herd.

Susceptible hosts include: Cattle, Sheep and Mares.

In the Northern Hemisphere, the incidence of mycotic abortion is highest between November and April, which corresponds to the approximate time when gravid cows are housed indoors and fed hay and/or ensilage. The disease can also occur in beef cattle confined to pens and fed hay as well as those on pasture. Furthermore, cows confined to sheds and fed in cubicals are at greater risk than those fed loose hay in an "open"barn.

Factors influencing susceptibility

- It is assumed that housing of animals in relatively confined spaces predisposes them to infection due to the presence of higher concentrations of fungal spores in the air of cowsheds than that of its surroundings (Turner, 1965).
- Pregnancy in a cow with metabolic derangements from stress may predispose the pregnant cow to fungal infection (Dalling, 1966).
- The incidence of the condition is high in late summer or early autumn, due to the presence of large number of fungal spores in pastures during this period (Stableforth and Galloway, 1959). There is also evidence of a winter rise of disease incidence.

Pathogenesis

- Principal entry of fungi is via the respiratory tract and the route of infection is via the blood stream in the lungs.
- Granulomatous lesions in the lungs could break down under stress, leading to invasion of blood vessels with hyphae.
- Small ulcers in the forestomach and abomasum in the cattle are well known and these may become invaded by the fungi.
- Spread of infection to the blood stream from such ulcers leads to either pneumonia or placentitis (Roberts, 1971).

Symptomatology

- In the experimental disease, a period of about one month lapses between intravenous inoculation of fungal spores and abortion, but natural incubation period is unknown.
- No noticeable symptoms have been recorded in the dam either before or after explusion of dead foetus.
- A tentative clinical diagnosis can be made on the pathological appearance of placenta and particularly the cotyledons and also on the presence of foetal skin lesions.

Lesions

- The placenta shows characteristic changes. The placental lesions are chiefly concerned with the adherence of maternal part of cotyledon to the chorionic part so that these organs appear as raised, solid, yellowish, cushion-like structures, often with a raised and thickened margin.
- Occasionally, the foetus shows skin lesions in the form of diffused white hair on the flanks, neck, axilla and inside the backs.
- Histological examination of the affected cotyledons shows extensive hyperaemia and haemorrhages in the early infection with scattered infiltration of polymorphonuclear leukocytes and eosinophils.

Diagnosis

Criteria for diagnosis

- A diagnosis of mycotic abortion was made when mycotic elements were seen associated with placentitis, fetal dermatitis, or pneumonia.
- Also, morphologic and pigmentation characteristics of hyphae seen in tissues had to be compatible with cultured isolates.
- A provisional field diagnosis can be made by the sporadic nature of the disease, with appearance of placental and foetal skin lesions.
- Abortions usually occur late in pregnancy and the palcenta is usually retained.
- Confirmation of mycotic abortion is made by microscopical and cultural examination.
- Hyphae may be detected by direct examination of wet preparations of affected

cotyledons and abomasal contents.

- The fungi are isolated from abomasal contents and cotyledons.
- Foetal stomach contents provide more useful material for culture and produce a pure growth of causative organisms.

Specimens

In some instances, fetal placenta and abomasal content but no other fetal tissues were available. Occasionally, formalin-preserved fetal and placental tissues along with abomasal content or only formalin-fixed tissues were examined.

Gross examination

For the diagnosis of mycotic infection, a sterile disposable syringe was used to aseptically collect l–3 ml of abomasal content. In addition, a piece of placenta with 2 or 3 cotyledons was placed in a sterile plastic bag, and depending on the availability of fetal tissues, portions of lung, eyelid, and skin (if there was evidence of dermatitis)were collected.

Mycological examination.

- Portions of placenta containing 2 or 3 cotyledons were placed in a beaker, washed in running tap water l–3 min to remove extraneous debris, then blotted with sterile paper towels.
- A sterile scalpel was used to scrape a small amount of material from the cut surface of all cotyledons, caruncles, and any fetal skin that had gross lesions.
- This material fetal lung that had been macerated with a sterile mortar and pestle, and 0.2 ml of fetal abomasal content were separately spread onto the surface of plates of Sabouraud Dextrose Agar (SDA) containing 1000 units/ml of Penicillin-G. Solid media were incubated at both room temperature and 37°C.
- Thereafter, primary isolation was performed only at 37°C. Plates were examined daily for the first week and twice each week for the next 2 weeks.
- Portions of detectable mycotic growth were transferred to Potato Dextrose Agar (PDA) and SDA plates and slants.
- PDA was used as a growth medium for slide cultures, which were incubated 3–7 days and then stained with lactophenol cotton blue.
- Identification of filamentous isolates was made from gross and microscopic characteristics.
- Yeasts were identified from carbohydrate assimilation patterns.

Direct microscopic examination

A small portion of each placental tissue, fetal skin with gross lesions, or abomasal content was placed on a glass slide with a drop of 10% potassium hydroxide, heated slightly over an open flame, and examined for mycotic elements by light microscopy.

Histologic examinations

Portions of placental cotyledons, intracotyledonary tissue with gross lesions, caruncle, fetal eyelid, skin with gross changes, and parenchymatous organs were fixed in 10% phosphate-buffered formalin, pH 7.0. Tissues were embedded in paraffin, sectioned 7 μm thick, and stained with hematoxylin and eosin. Gomori methenamine silver and periodic acid (Schiff's) stains were used to stain mycelial elements in tissue sections.

Isolation and Identification

A. fumigatus isolation is carried out on Sabourauds dextrose agar without cyclohexamide. The cultures are incubated aerobically at 37^0C for upto 5 days. A.fumigatus colonies rapidly become velvety and granular and bluish green with narrow white pheriperes. Older colonies are slate-grey.

Absidia, Mucor and Rhizopus

Growth of Absidia, Mucor and Rhizopus species is rapid, filling the petridish with greyish or brownish - grey fluffy colonies within a few days.

Absidia corymbifera: Rapid growth, wooly and white becoming olive grey. Fills petridish like Rhizopus. It is thermotolerant and grows at 45°C.

Mucor: Colonies spread right across petridish but growth is low. Pale grey or yellowish brown at 37°C

Rhizopus: Coarse, rampant growth. Petridish is filled in 5 days with dense, wooly mycelium. White at first, becoming greyish and surmounted by black pin-head-sized sporangia.

Rhizomucor pusillus: Rapidly growing, cottony colonies. White but surrounded by brown

sporangia. Thermophilic with growth at 50°C–60°C.

Mortierella wolfi: M. Wolfi has characteristic white velvety colonies with lobulated outlines giving a rosette appearance colonies are about 5 cm in diameter after incubation for 4 days.

C. albicans: Isolation out on Sabourauds dextrose agar with cyclohexamide. Colonies are whitish, shiny and convex 4 to 5 mm in diameter after incubation for 3 days at 37°C

Differential diagnosis

Diagnosis of mycotic abortion presents great difficulties because a number of infectious and non-infectious agents are known to cause abortion in cattle. Abortion resulting from various infectious causes must be differentiated from mycotic abortion. Confirmation lies in the isolation of specific etiologic agent.

Prognosis

There is conflicting evidence regarding the effect of uterine fungal infection on the subsequent breeding performance of the cow. Resumption of regular breeding is certainly not ruled out by mycotic abortion, but there is not sufficient information to estimate the future performance of an affected cow.

Treatment

No clinical symptoms have been observed in the dam either before or after abortion and no treatment has ever been given to the affected animals.

Control

- Since the epizootiology of mycotic abortion is obscure, evidence on the methods of control is speculative.
- If mouldy hay and straw are assumed to be the commonest source of infection, a careful watch on the quality of these materials is essential, so that any sample that appears excessively dusty may be rejected.
- Dust has been shown to consist chiefly of fungal spores of various types, but more especially the spores of mycotic abortion.
- Treatment of hay with some suitable fungicide during haymaking should be done in order to reduce subsequent mould growth.
- Housing of animals in relatively confined spaces should also be avoided because some evidence indicates that air of over-crowded cowsheds is rich in spores of fungi and can cause abortion.

References

1. Ali R and Khan IH. Mycotic abortion in cattle. Pakistan Veterinary Journal, 2006;26(1):44–46.
2. Carter ME, Cordes DO, Di Menna ME and Hunter R. Fungi isolated from bovine mycotic abortion and pneumonia with special reference to Mortierella wolfii. Research in Veterinary Science, 1973;14:201–06.
3. Dalling ST. International Encyclopedia of Veterinary Medicine. IV: W. Green & Sons. Ltd. Edinburgh, UK,1966. pp:1970–1972.
4. Jenson HE, D. Stynen and Sarfati. Detection of galactomannan and 18 KDa antigen from Aspergillus fumigatus in serum and urine. Zentralbl Veterinarmed B., 1993;40(6):397-408.
5. Knudtson WU and Kirkbride CA. Fungi associated with bovine abortion in the northern plain states (USA). Journal of veterinary Diagnsotic investigation, 1992;4:181–85.
6. MacDonald SM and Corbel MJ. Mortierella wolfii infection in cattle in Britain. Veterinary Record, 1981;109:419–21.
7. Roberts SJ. Veterinary Obstertics and Genital Diseases. Edward Brothers, Inc. Michigan, USA, 1971;85–86.
8. Stableforth WA and Galloway AI. Infectious Diseases of Animals. Vol. I: Butterworths Scientific Pub., London, UK, 1959;287–88.
9. Theobald Smith. Mycosis of the bovine fetal membranes due to a mould of the genus Mucor. J. Exp. Med., 1920;31:115–22.
10. Turner PO. Association of fungi with mycotic abortion in Hong Kong. Vet. Res., 1965;77(10):273–76.
11. Quinn PJ, Markey BK, Carter ME, Donnelly W.J and Leonard FC. Veterinary Microbiology and Microbial Disease. 2002.
12. Quinn PJ, Carter ME, Markey BK and GR. Carter. Clinical Veterinary Microbiology. 1999.

Review Article

Journal of Animal Feed Science and Technology
Volume 7 Number 2, July – December 2019
DOI: http://dx.doi.org/10.21088/jafst.2321.1628.7219.6

Heat Applications in Feed and Food Processing

Yavuz Gurbuz

Professor, Department of Animal Nutrition, Faculty of Agricultur, University of Kahramanmaras Sutcu Imam , Campus of Avsar Kahramanmaraş, Turkey.

How to cite this article:
Yavuz Gurbuz. Heat Applications in Feed and Food Processing. Journal of Animal Feed Science and Technology. 2019;7(2):93–98.

Abstract

There are many heat treatment applications, each different in the heat source, construction of the device or process parameters applied, and their efficiency depends on a range of factors. There are many possible combinations, and types of heat treatments in animal feed processing, and most frequently used are pelleting, extrusion, cooking, steam flaking, conditioning, expanding, roasting, popping, toasting and micronisation. Thermal processing increase feed intake and digestibility, improve feed conversion, carcass quality and/or yield grade, reduce in feed waste, transportation, storage costs and labor costs. The heat acts in the same manner as natural digestive enzymes to break down the complex carbohydrate bonds of the grain starch (i.e. gelatinize), which increases the availability of nutrients such as glucose in the small intestine. However, the heat technology used can destroy germs contaminating the oilseeds and remove certain anti-nutritional (antitrypsin factors in whole soya beans, rapeseed glucosinolates) and enzymatic factors (lipases and lipoxidases involved in the oxidation of oils for example).

Keywords: Thermal processing; Heat applications; Feed and food processing.

Introduction

Feed processing techniques have changed over time and now require greater precision. Today, livestock production must meet new demands from consumers, especially where the nutritional value of feed is concerned. Feed may look different varying from pellets to flaked grains and extruded nuggets depending on the processing method, but is this for visual reasons or are there nutritional purposes[1,2]. All questions will be answered in this short article, highlighting the three main processing techniques, we use here at the hygiene animal only feed mill, its differences and various benefits. Goals of heat treatment are increased feed intake and digestibility, improved feed conversion or efficiency, improved carcass quality and yield grade, reduction in feed waste, lower transportation and storage costs, reduced labor costs due to increasedmechanization.

The heat treatment under certain conditions of temperature, time and moisture, allows the elimination of salmonella in food. Moreover, the improving of the flow reduces the possibility of bacterial growth by preventing the retention and accumulation of the product (Fig. 1).

Corresponding Author: Yavuz Gurbuz, Professor, Department of Animal Nutrition, Faculty of Agricultur, University of Kahramanmaras Sutcu Imam , Campus of Avsar Kahramanmaraş, Turkey.

E-mail: yavuz@ksu.edu.tr

Received on 01.10.2019; **Accepted on** 13.11.2019

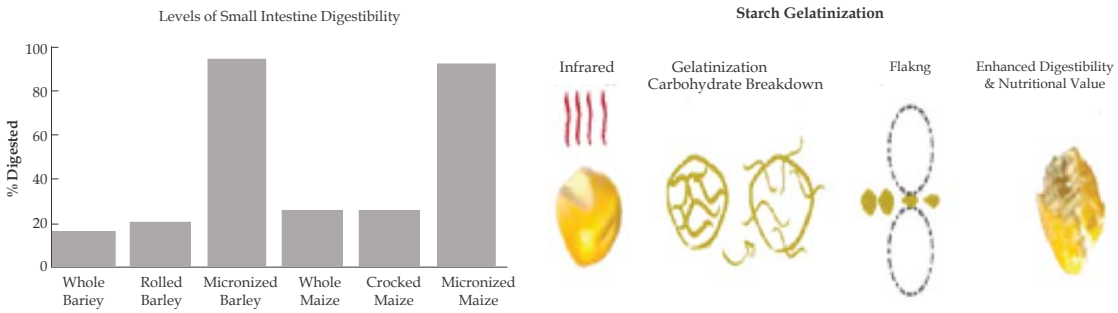

Fig. 1: levels small intestine digestibility and starch gelatinization in heat treatment[3]

Feed ingredients and animal feed are frequently contaminated with foodborne bacterial pathogens like campylobacter species and non-typhi serotypes of salmonella enterica, Toxin–producing strains of escherichia coli, and yersiniaenterocolitica.[4–7] Thermal processing of feed can improve the stability and hygiene of feed, might alter the chemical and physical characteristics of its constitutive ingredients, can improve the nutritional value of animal feeds. So thermal processing might have beneficial effects on the gastrointestinal function and the microbial status of gastrointestinal tract.[8,9]

In order to eliminate, inactivate or reduce the anti- nutrients and pathogens in the animal feed ingredients and feed, a simple heat processing method have been used for decades. This simple heat processing method could be dry or moist, and based on the purposes of the usage and its technical characteristics can be classified as cooking, roasting, toasting, autoclaving and long term conditioning. Grains are process or treat by mechanical, thermal or thermo-mechanical methods into dry or wet. Factors affecting starch digestion include the level of intake, the actual ingredient (e.g. oats, barley, maize) and how the grain was processed. The processing of grains is applied to maximize the small intestine digestibility of the grain in order to reduce the risk of digestible disorders whilst increasing the nutritional value of the feed.

Types of Heat Applications

Thermal processing are used to improve the nutritional, hygienic, physical and chemical of animal feed properties. The two most important factors on all heat treatments are temperature and time of their application, although the impacts such as humidity, pressure. Most of heat treatments that are used are hydro thermals because even when moisture is not introduced from the outside moisture released from the material to be treated participates in heat application. Another effect of factorsis mechanical and it can be located in or out of the heat processing device. The mechanical treatment causes an additional effect on animal feeds. Starch granules undergo gelatinization and melting by the action of heat and moisture onhydrogen binding among tightly packed polysaccharide chains in the granule structure. Under conditions of excess water, hydrogen bindings in the less ordered amorphous regions of the granule are disrupted first, allowing water to associate with free hydroxyl-groups. Swelling is the result and further opening of the granule structure to the action occurs. Thus, there are many possible combinations, and types of heat applications in animal feed processing. Most commonly used thermal methods in the world are pelleting, expanding, extrusion, steam flaking, conditioning, cooking, micronisation, roasting, popping, toasting e.g.).[10,11]

Pelleting

Pellet units are one of the technological processes in feed mill and it can be defined as a collection of single ingredients or combinations by pressing and making it through openings in the die and cutting off of the pressed feed forms to the preferred length. It is typical to condition animal feed before pelleting. In this technique, the temperature of the pellet feeds usually increases to about 70°C–80°C before inflowing the press matrix for pelleting. Supplementary heating is reached during the pelleting process by the use of mechanical act of power, two or more rollers, with the same or different diameters, which rotate sideways a horizontal die, or inside the circle die. Rollers pass over the mash feed and compress it. The pressure continuously increases from the point where the rollers touch the mash feed and start pushing it towards the hole on the die to the point where the pressure is big enough to insert a small disc of feed hooked on the opening of the die canal and unite which was already in it. The pressure extents its extreme at the point where the gap among the roller and the die is the least, so

that a part of the shaped pellet is hard-pressed out on the other sideways of the die opening. Forces can be enhanced by growing the pressure, which causes the rise in heat of the product as well.The selection of pelletizing press features, grinding, conditioning is possible to effect the saving of friction, or an rise of adhesion matrix that bind the particles in the pelleting procedure, and thus the quality of shaped pellets that are expressed as % of friction or hardness and consumption of press power essential to accomplish the vital pressure through the fitted power of electric engine.[4,13] The increased humidity and temperature are removed in the cooling procedureof vertical or horizontal coolers. It is essential to decrease these sizes into the storing stability. The developments of this pellet process tend towards advanced automation, which should allow continuous control and alteration of progression variables, its higher productivity, and better pellet feeds quality (chemical, nutritional, microbiological, physical e.g.).

Expansion

The extrusion and expansion methods are based on the similar principles. Fundamentally, the expanders are very like to extruders, and they vary in the method of forming of the last product and force of treatment.[14,15] Expanders are generally used as mechanical conditioners for feed process which are difficult to pelleting of feed, to improve the digestibility of cellulose and protein in order to make feed hijyenisation.[17]

Extrusion

The extrusion process begins with the grinding and mixing of ingredients such as grains, oil and fibresources. Extrusion is the procedure in which the feedstuff and compound feed is hard-pressed through the barrel by means of screws of altered formations and pressed through the die at the end of extrusion barrel. The basic model of extrusion procedure is high temperature, short time, whereby the high temperature is a direct outcome of dry extrusion or preconditioning and wet extrusion (steam addition), or a mixture of both. The moisture of treated feed and food in dry extrusion is about 30% while it is up to 80% in wet extrusion. Extruders can be sterilization as those with one or two screws, and the latter may have screws that are rotating in the similar or in contrary directions, and screws can also narrow in a conical form. Extrusion is the procedure in which the feed and food are exposed to high heats (up to 200 °C) for 1–2 minutes or more accurately the feed and food temperature risessuddenly within the last 15 to 20 seconds up to the optimal one to do the preferred effects. Thus, this procedure is categorized as heat treatment with high temperatures and short period of its action. The feed and food for extrusion are also exposed to reasonably high pressure, which can range up to 25 Mpa. The pressure difference between the inside of the extruder and the outside situation reasons part evaporation of water at the exit point, and thus the expansion of the feed and food. It is possible to do a range of special effects on the treated feed and food, such as grinding, hydration, cutting, sterilization, mixing, dispersion, compression, heat treatment, and inactivation of anti-nutritional substances, compression, and expansion,

binding of particles, formation of porous structure and partial dehydration and sterilization. The kind and force of induced variations depend on the added energy in relative to time and quantity of feed and food, design of screws (spiral shape, segments for slowing down, type and length of individual segments, the ratio between the length and diameter), type and structure of feed and food to be treated, humidity and fat content, capacity, additional heating and cooling of each barrel section, and die geometry.[16,17] Extrusion is a complex and complicated technicalprocedure, but it is very elastic and offers the possibility for processing of a range of different of stuff, feed and food.[8,9,17]

Steam flaking

Flaking is the procedure in which the grain feed are exposed to special effects of water steam in the situations of atmospheric or high pressure, and then rolled to get hold of thin sheets. The thickness of flakes is defined by correcting the spacing between the rollers and it ranges from 0.4 to about 2.0 mm. There are many differences of this process, depending on the pressure and temperature values and period of the process. Steam flaking is used as a heat treatment of all types of grains and cereals suchas corn, barley, wheat, etc. Moisture content in grain, thickness of flakes, grain temperature, and period of the progression affect the procedure efficiency. Grain heat during the treatment reaches about 100°C.[11,12,17]

Conditioning

Conditioning is a common term for procedures in which the material is prepared for the following industrial process. Conditioning commonly suggests preparation of raw materials or mixtures for pelleting or extruding and expanding in feed mill. Procedures of conditioning are water conditioning, steam conditioning (short and extended) and

mechanical conditioning. So, conditioning improved physical quality of the feed and food. The possibility to treat more feed and food materials Increased hygienic correctness of foodstuffs.[9,17] The simplest technique of conditioning is to add water into the conditioning machine. Humidity and heat are accomplished in a more effective way in steam conditioning procedure. Due to its vaporous state, steam spreads through the material in a more homogenous way. This procedure is carried out by direct injection of dry saturated steam into the feed and its heat can reach 95°C. The procedure of steam heating is restricted because it sources a rise in moisture content of the food and feed for 1% for every 12–15°C of temperature increase. The required condition for worthy conditioning is dry saturated steam pressure of about 8–10 bar with the temperature of 150–180°C.[12,11] Decrease of pressure on the spot of use by around 1.5–3.0 bar reasons steam temperature decline and the released heat or overheats it, if there is no condensate in it. In this way, the over-wetting of feed is evaded and it is heated in the most capable way. Using a lower steam pressure more moisture is presented in the feed for the same heating level (9;10;11). A thin water film is formed around a particle, which together with the increased heat facilitates binding of particles in during the steam condensation,. The main factors in conditioning contain the temperature, moisture content and treatment period. Temperature and moisture quantity are gotten by adding steam, and the time factor depends on the kind, size and functioning of the device. The device for short-term conditioning is a non-stop paddle mixer to which water or dry saturated steam is supplemented. The feed and food are conveyed through the feeder with variable rotation speed. First particles of the feed leave the mixer in only a few seconds and that time is not enough to use all the conditioning capacities. The average time of holding feed in this form of conditioner is 10 to 30 seconds. Chambers for lengthy conditioning allow better diffusion of moisture, and heat into feed and food particles.[9,17] Steam conditioning is not enough to achieve the satisfying pellet quality when feed of poor bonding properties are pelleted. Therefore, a combination of steam conditioning and mechanical conditioning is applied. Thickness of material layer on the die and rise of engine power of electric motors in thicker die, are theways to extend conditioning of feeds in the procedure of pelleting. Expander or extruder are fixed as special systems for mechanical conditioning in order to heat up the material to 100–140° and even up to 170°C before final pelleting. An increased consumption of energy is predictable, so that the application of this style of procedure is justified only in cases when the last price of the product can stand funds in equipment and production.[17,19]

Cooking

Raw seed are saturated in water and heated for 30 to 120 minutes, and then they are dried, and given to animals as feed, whole, milled, or rolled. Cooking is a comparatively easy to make technique. Pressure cooking is a difference of this procedure, when the management is carried out in closed containers under the pressure of steam that is produced. In this way, we can success heats higher than 100°C. These procedures have restricted use because they are not flexible adequate.[17,20] Hot-headed cooking is the cooking method in which raw feed is heated by steam. It takes place in the containers under the pressure of 2.3 to 3.0 MPa. Opening of the container upon end of treatment creates a sudden pressure loss in the container due to balancing by atmospheric pressure and make available for extra expansion of grain and extra influence on the treated feed. The procedure of explosive cooking is much more flexible than the formerly stated styles of cooking. This procedure can accomplish a wide range of different heats and pressures and is suitable for giving of all styles of granular raw materials.[8–10,17]

Micronisation

Grain can be heated by a variation of procedures that use spread of the waves, which vary in part of the electromagnetic spectrum that is used. Micronisation is a specific heat treatment in which the layer of grain on the conveyor belt is constantly carried below ceramic radiators emitting radiation with wavelength in the near infrared region ranging from 1.8 to 3.4 cm. The spread rays, which are engaged to a product, cause the frequencies from 80 to 170 million mega beets per second inside the grain, which leads to rapid heating, bigger stress of water vapour and rapid water evaporation. Micronisation reductions the moisture content of grain by 30–40%. The concentration of infrared rays' translation into heat and its influence depends on the type of feed to be treated.[21]. The conveyor belt within the microniser can waver in order to tumble the grain and expose well all its surfaces to waves influence. The most vital parameters of this treatment are the speed of the conveyor belt, thickness of product layer, space between the product and the radiation basis and certainly the accomplished temperature.[2,12,17] During the micronizing process, the grain is heated

using infrared heat until all moisture is vaporized (generally less than a minute). This ruptures the endosperm of the grain, leading the grain to become soft and pliable, causing the reconfiguration of the starch structure (gelatinization). Immediate flaking further gelatinizes the starch so as to significantly enhance the digestibility and nutritional value of the feed.

Roasting

Roasting is difficult dry heating of raw feed and food to the temperature of 110–170°C, depending on the style of device used and the wanted product value. If the roasting temperature is too high, it decreases the availability of nutrients in the shallow layers of grain, while the central portion may stay under-treated. The lower temperature reduces the risk of burning out and burning, but it also decreases the capability of the device. Many different systems of roasting are used all over the world. Most of these systems contain a throughinfluence of heat on kernel, and due to a direct contact with the grains of different types and sizes, the quality, stability and degree of roasting as well as grain colourcan differcommonly.[2,10,11,17] The simplest way of roasting of soybeans and other granular raw materials for animal feed is roasting in different types of dryers. The most commonly used structures are those that are based on rotating drum- type dryers because they are suitable for unimportant funds, and easy to handle, and as they do not need any great space to accommodate even large supporting installations. The grain in these devices is commonly heated directly by hot air heated by burning gas, solid or liquid fuels. The product is mixed by barrel rotation and fixed blades in its inside. Some apparatus of this type use microwave radiation in mixture with direct heating by hot air stream.[11,16] Conveyer dryers using air heated byheatexchangers as liquid are also used for roasting. The advantage of this type of device is that the grain is not exposed to direct flame and combustion products. A newer high efficacy drying technology of 13 fluidized bed type use dry overheatedair that is blown through the grain and that has the product in a permanent suspension and effort below the organized temperature and time of product retention. The grain is "cooked" by its own moisture, and this procedure provides a very hygienic product of uniform high value. The production hot air can be recycled, dried, and re-used thus growing the economic efficacy of the procedure.[2,9–11,17]

Popping

Popping is the method of roasting of dry grain on a hot plate (t < 400°C) in a short time. Such treatment of grain leads to quick loss of moisture, grain exploding into popcorn and increasing of its nutritive value. All varieties of grain can be applied this method, and it is the best to use this treatment on corn since the lowest share of un-popped grains is well known.[17] Rolling can be carried out after popping to rise the bulk density of the feed andfood.[2,21]

Toasting

Toasting that is introduced straight into the toasting vessel is a hot fluid in this procedure. The vessel may have different structures and it can have the units through which the produce passes. The treatment is the one with the time of holdingwithin the vessel from 10 to 20 minutes and the released temperature of up to 120°C. After heat action, mechanical pressure between two rollers can be used to form flakes, and then the product is cooled. This process can be useful to treat several types of raw materials. Common is its use in edible oil production, where it is used to extract the solvent from the meal after oil extraction. This process can be preferred due to decrease of anti-nutritive substances.[2,11,12,17]

Conclusion

Mash feed compound, soybeans and oilseeds or legumes provide a good example of improved protein digestibility andbioavailability of sulphur amino acids through thermal unfolding of the major globulins, and thermal inactivation of trypsin inhibitors and other growth-retarding factors such as lectins. However, extensive lysine loss can take place when legume or cereal legume blends are extrudedunder severe conditions of temperature or shear forces (>l00 rpm) at low moisture (<15%), especially in the presence of reducing sugars (23% glucose, fructose, maltose, lactose). This damage depends on the maillard condensation between &-NH_2 groups of lysine residues and C=O groups of reducing sugars. It is not fully understood whether the damaging effects. The nutritional value of lipids could be affected during extrusion as a result of oxidation, hydrogenation, isomerization or polymerization. The autoxidation of essentialfatty acids (linoleic, linolenic, arachidonic) renders them unable toprevent the dermatitis and poor growth associated with low intake of these nutrients. lsomerization of the double bonds from the cis to the trans form also destroys the essential activity of these PUFA. However, the amount of hydrogenation and cis-trans isomerization of

fatty acids that takes placeduring extrusion is too small to be nutritionally significant. Extrusion-inactivation of lipase and lipoxidase helps protect against oxidation during storage.

Higher temperatures reduce the lipase activity and moisture level, thereby decreasing favoring freefatty acids development. However, the expanded porous nature of the extrudate causes the feed tobe susceptible to the development of oxidation during storage, even though deterioration due toextrusion may not be immediately apparent. Dietary fiber; Modifications in particle size, solubility and chemical structure of the various fiber components could occur during extrusion-cooking and cause changes in bacterial degradation in the intestine andin physiological properties. Extrusion-cooking of white wheat flour (161–171°C; 15% moisture) was found to cause are distributionof insoluble to soluble dietary fiber. However, food and feed processing industry is to minimize the loss of nutrients during thermal processing while providing an adequate process.

References

1. Gurbuz Y, Demir OL, Furkan Y, et al. Effects of heat processing on nutritive value of cottonseed. XVI Intetnational Symposium 'Feed Techology'. 2014. pp.245–52.
2. Ari MM, Ayanwale BA, Adama TZ. et al. Evaluation of the Chemical Composition and Anti Nutritional Factors (ANFs) Levels of Different Thermally Processed Soybeans. Asian Journal of Agricultural Research. 2012;6:91–98.
3. Auriane H. 2015. Feed processing: to help not harm.http://www.hygain.com.au/micronizing-improved-feed- efficiency.
4. Gurbuz Y, Zincirlioğlu M, Alarslan ÖF. Effecting Factors Of Quality of Pellet Feeds Using in Animal Nutrition. 1998.pp.224–26. Tuyem IV. Kapodokya, Turkey.
5. Gurbuz Y, Yazgan O, Kamalak A. Effects of Different Pellet Binders on Pellet Quality of Compound Feeds. KSU J. Science and Engineering 2003;6(1):160–68.
6. Muramatsu K, Maiorka A, Dahlke F, et al. Impact of Particle Size, Thermal Processing, Fat Inclusion and Moisture Addition on Starch Gelatinization of Broiler Feeds. Rev. Bras. Cienc.Avic. 2014;16(4):367–74.
7. Thomas M and Van der Poel AFB. Physical Quality of Pelleted Animal Feed: Part 1, Criteria for Pellet Quality. Anim. Feed Sci. Technol. 2000;61:89–112.
8. Goelema JO, Smits A, Vaessen LM, et al. Effects of Pressure Toasting, Expander Treatment and Pelleting on in Vitro and in Situ Parameters of Protein and Starch in a Mixture of Broken Peas, Lupins and Faba Beans.Anim. Feed Sci. Technol. 1999;78(1–2):109–26.
9. Abdollahi MR, Ravindran V, Wester TJ, et al. Effect of Improved Pellet Quality from the Addition of a Pellet Binder and/or Moisture to a Wheat-Based Diet Conditioner at Two Different Temperatures on Performance, Apparent Metabolisable Energy and Ileal Digestibility of Starch and Nitrogen in Broilers. Anim. Feed Sci. Technol. 2012;175(3–4):150–57.
10. Abdollahi MR, Ravindran V and Svihus B. Pelleting of broiler diets: An overview with emphasis on pellet quality and nutritional value. Animal Feed Science and Technology 2013;179(1):1–23.
11. Jansen HD. Extrusion Cooking for mixed Feed Processing, Advances in Feed Technology, 1991;5,s:58–66.
12. Riaz MN. Extruders and expanders in Pet Food, Aquatic and Livestock Feeds, Agrimedia GmbH, Clenze, Germany, 2007.p.387.
13. Engelen GMA. Post-pelleting application of liquid additives, Wageningen Pers, Wageningen, Netherlands 1999.
14. Poel AFB. Expander Processing of animal Feeds, Feed Processing Centre, Wageningen, 1997.
15. Riaz MN. Extruders and expanders in Pet Food, Aquatic and Livestock Feeds, Agrimedia GmbH, Clenze, Germany, 2007.p.387.
16. Heindreich E. Increasing products safety by expanding technology, Feed Tech, 2002;6(9/10):9–11.
17. Jovanka L and Sredanovic S. Heat Treatments in Animal Feed Processing XVI Intetnational Symposium 'Feed Techology' 2014.pp.1-25, Novi Sad, Serbia.
18. Kirchner A. Expanding & Extrusion, Fundamentals and new developments in feed-compounding technology, Braunschweig-Thune, Germany 2009. pp.1–18.
19. Cox NA, Burdick D, Bailey JS, et al. Effect of the Steam Conditioning and Pelleting Process on the Microbiology and Quality of Commercial-Type Poultry Feeds, Poultry Science, 1986;65(4),704–09.
20. Monari S. Full-fat Soya Handbook,www.asasea.com
21. Sakač M, Ristić M, Lević J. Effects of Microwave Heating on the Chemico-nutritional Value of Soybeans. Acta Alimentaria 1996;252:163–69.

Subject Index

Author Index

Guidelines for Authors

Manuscripts must be prepared in accordance with "Uniform requirements for Manuscripts submitted to Biomedical Journal" developed by international committee of medical Journal Editors

Types of Manuscripts and Limits

Original articles: Up to 3000 words excluding references and abstract and up to 10 references.

Review articles: Up to 2500 words excluding references and abstract and up to 10 references.

Case reports: Up to 1000 words excluding references and abstract and up to 10 references.

Online Submission of the Manuscripts

Articles can also be submitted online from http://rfppl.co.in/customer_index.php.

I) First Page File: Prepare the title page, covering letter, acknowledgement, etc. using a word processor program. All information which can reveal your identity should be here. use text/rtf/doc/PDF files. Do not zip the files.

2) Article file: The main text of the article, beginning from Abstract till References (including tables) should be in this file. Do not include any information (such as acknowledgement, your name in page headers, etc.) in this file. Use text/rtf/doc/PDF files. Do not zip the files. Limit the file size to 400 Kb. Do not incorporate images in the file. If file size is large, graphs can be submitted as images separately without incorporating them in the article file to reduce the size of the file.

3) Images: Submit good quality color images. Each image should be less than 100 Kb in size. Size of the image can be reduced by decreasing the actual height and width of the images (keep up to 400 pixels or 3 inches). All image formats (jpeg, tiff, gif, bmp, png, eps etc.) are acceptable; jpeg is most suitable.

Legends: Legends for the figures/images should be included at the end of the article file.

If the manuscript is submitted online, the contributors' form and copyright transfer form has to be submitted in original with the signatures of all the contributors within two weeks from submission. Hard copies of the images (3 sets), for articles submitted online, should be sent to the journal office at the time of submission of a revised manuscript. Editorial office: Red Flower Publication Pvt. Ltd., 48/41-42, DSIDC, Pocket-II, Mayur Vihar Phase-I, Delhi – 110 091, India, Phone: 91-11-22754205, 45796900, 22756995. E-mail: author@rfppl.co.in. Submission page: http://rfppl.co.in/article_submission_system.php?mid=5.

Preparation of the Manuscript

The text of observational and experimental articles should be divided into sections with the headings: Introduction, Methods, Results, Discussion, References, Tables, Figures, Figure legends, and Acknowledgment. Do not make subheadings in these sections.

Title Page

The title page should carry

1) Type of manuscript (e.g. Original article, Review article, Case Report)

2) The title of the article should be concise and informative;

3) Running title or short title not more than 50 characters;

4) The name by which each contributor is known (Last name, First name and initials of middle name), with his or her highest academic degree(s) and institutional affiliation;

5) The name of the department(s) and institution(s) to which the work should be attributed;

6) The name, address, phone numbers, facsimile numbers and e-mail address of the contributor responsible for correspondence about the manuscript; should be mentoined.

7) The total number of pages, total number of photographs and word counts separately for abstract and for the text (excluding the references and abstract);

8) Source(s) of support in the form of grants, equipment, drugs, or all of these;

9) Acknowledgement, if any; and

l0) If the manuscript was presented as part at a meeting, the organization, place, and exact date on which it was read.

Abstract Page

The second page should carry the full title of the manuscript and an abstract (of no more than 150 words for case reports, brief reports and 250 words for original articles). The abstract should be structured and state the Context (Background), Aims, Settings and Design, Methods and Materials, Statistical analysis used, Results and Conclusions. Below the abstract should provide 3 to 10 keywords.

Introduction

State the background of the study and purpose of the study and summarize the rationale for the study or observation.

Methods

The methods section should include only information that was available at the time the plan or protocol for the study was written such as study approach, design, type of sample, sample size, sampling technique, setting of the study, description of data collection tools and methods; all information obtained during the conduct of the study belongs in the Results section.

Reports of randomized clinical trials should be based on the CONSORT Statement (http:// www. consort-statement. org). When reporting experiments on human subjects, indicate whether the procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional or regional) and with the Helsinki Declaration of 1975, as revised in 2000 (available at http://www.wma. net/e/policy/l 7-c_e.html).

Results

Present your results in logical sequence in the text, tables, and illustrations, giving the main or most important findings first. Do not repeat in the text all the data in the tables or illustrations; emphasize or summarize only important observations. Extra or supplementary materials and technical details can be placed in an appendix where it will be accessible but will not interrupt the flow of the text; alternatively, it can be published only in the electronic version of the journal.

Discussion

Include summary of key findings (primary outcome measures, secondary outcome measures, results as they relate to a prior hypothesis); Strengths and limitations of the study (study question, study design, data collection, analysis and interpretation); Interpretation and implications in the context of the totality of evidence (is there a systematic review to refer to, if not, could one be reasonably done here and now?, What this study adds to the available evidence, effects on patient care and health policy, possible mechanisms)? Controversies raised by this study; and Future research directions (for this particular research collaboration, underlying mechanisms, clinical research). Do not repeat in detail data or other material given in the Introduction or the Results section.

References

List references in alphabetical order. Each listed reference should be cited in text (not in alphabetic order), and each text citation should be listed in the References section. Identify references in text, tables, and legends by Arabic numerals in square bracket (e.g. [10]). Please refer to ICMJE Guidelines (http://www.nlm.nih.gov/bsd/uniform_ requirements.html) for more examples.

Standard journal article

[1] Flink H, Tegelberg Å, Thörn M, Lagerlöf F. Effect of oral iron supplementation on unstimulated salivary flow rate: A randomized, double-blind, placebo-controlled trial. J Oral Pathol Med 2006; 35: 540–7.

[2] Twetman S, Axelsson S, Dahlgren H, Holm AK, Källestål C, Lagerlöf F, et al. Caries-preventive effect of fluoride toothpaste: A systematic review. Acta Odontol Scand 2003; 61: 347–55.

Article in supplement or special issue

[3] Fleischer W, Reimer K. Povidone-iodine antisepsis. State of the art. Dermatology 1997; 195 Suppl 2: 3–9.

Corporate (collective) author

[4] American Academy of Periodontology. Sonic and ultrasonic scalers in periodontics. J Periodontol 2000; 71: 1792–801.

Unpublished article

[5] Garoushi S, Lassila LV, Tezvergil A, Vallittu PK. Static and fatigue compression test for particulate filler composite resin with fiber-reinforced composite substructure. Dent Mater 2006.

Personal author(s)

[6] Hosmer D, Lemeshow S. Applied logistic regression, 2nd edn. New York: Wiley-Interscience; 2000.

Chapter in book

[7] Nauntofte B, Tenovuo J, Lagerlöf F. Secretion and composition of saliva. In: Fejerskov O,

Kidd EAM, editors. Dental caries: The disease and its clinical management. Oxford: Blackwell Munksgaard; 2003. pp 7–27.

No author given

[8] World Health Organization. Oral health surveys - basic methods, 4th edn. Geneva: World Health Organization; 1997.

Reference from electronic media

[9] National Statistics Online—Trends in suicide by method in England and Wales, 1979–2001. www.statistics.gov.uk/downloads/theme_health/HSQ 20.pdf (accessed Jan 24, 2005): 7–18. Only verified references against the original documents should be cited. Authors are responsible for the accuracy and completeness of their references and for correct text citation. The number of reference should be kept limited to 20 in case of major communications and 10 for short communications.

More information about other reference types is available at www.nlm.nih.gov/bsd/uniform_ requirements.html, but observes some minor deviations (no full stop after journal title, no issue or date after volume, etc.).

Tables

Tables should be self-explanatory and should not duplicate textual material.

Tables with more than 10 columns and 25 rows are not acceptable.

Table numbers should be in Arabic numerals, consecutively in the order of their first citation in the text and supply a brief title for each.

Explain in footnotes all non-standard abbreviations that are used in each table.

For footnotes use the following symbols, in this sequence: *, ¶, †, ‡‡,

Illustrations (Figures)

Graphics files are welcome if supplied as Tiff, EPS, or PowerPoint files of minimum 1200x1600 pixel size. The minimum line weight for line art is 0.5 point for optimal printing.

When possible, please place symbol legends below the figure instead of the side.

Original color figures can be printed in color at the editor's and publisher's discretion provided the author agrees to pay.

Type or print out legends (maximum 40 words, excluding the credit line) for illustrations using double spacing, with Arabic numerals corresponding to the illustrations.

Sending a revised manuscript

While submitting a revised manuscript, contributors are requested to include, along with single copy of the final revised manuscript, a photocopy of the revised manuscript with the changes underlined in red and copy of the comments with the point-to-point clarification to each comment. The manuscript number should be written on each of these documents. If the manuscript is submitted online, the contributors' form and copyright transfer form has to be submitted in original with the signatures of all the contributors within two weeks of submission. Hard copies of images should be sent to the office of the journal. There is no need to send printed manuscript for articles submitted online.

Reprints

Journal provides no free printed, reprints, however a author copy is sent to the main author and additional copies are available on payment (ask to the journal office).

Copyrights

Declaration

A declaration should be submitted stating that the manuscript represents valid work and that neither this manuscript nor one with substantially similar content under the present authorship has been published or is being considered for publication elsewhere and the authorship of this article will not be contested by any one whose name(s) is/are not listed here, and that the order of authorship as placed in the manuscript is final and accepted by the co-authors. Declarations should be signed by all the authors in the order in which they are mentioned in the original manuscript. Matters appearing in the Journal are covered by copyright but no objection will be made to their reproduction provided permission is obtained from the Editor prior to publication and due acknowledgment of the source is made.

Approval of Ethics Committee

We need the Ethics committee approval letter from an Institutional ethical committee (IEC) or an institutional review board (IRB) to publish your Research article or author should submit a statement that the study does not require ethics approval along with evidence. The evidence could either be consent from patients is available and there are no ethics issues in the paper or a letter from an IRB stating that the study in question does not require ethics approval.

Abbreviations

Standard abbreviations should be used and be spelt out when first used in the text. Abbreviations should not be used in the title or abstract.

Checklist

- Manuscript Title
- Covering letter: Signed by all contributors
- Previous publication/ presentations mentioned, Source of funding mentioned
- Conflicts of interest disclosed

Authors

- Middle name initials provided.
- Author for correspondence, with e-mail address provided.
- Number of contributors restricted as per the instructions.
- Identity not revealed in paper except title page (e.g. name of the institute in Methods, citing previous study as 'our study')

Presentation and Format

- Double spacing
- Margins 2.5 cm from all four sides
- Title page contains all the desired information. Running title provided (not more than 50 characters)
- Abstract page contains the full title of the manuscript
- Abstract provided: Structured abstract provided for an original article.
- Keywords provided (three or more)
- Introduction of 75-100 words
- Headings in title case (not ALL CAPITALS). References cited in square brackets
- References according to the journal's instructions

Language and grammar

- Uniformly American English
- Abbreviations spelt out in full for the first time. Numerals from 1 to l0 spelt out
- Numerals at the beginning of the sentence spelt out

Tables and figures

- No repetition of data in tables and graphs and in text.
- Actual numbers from which graphs drawn, provided.
- Figures necessary and of good quality (color)
- Table and figure numbers in Arabic letters (not Roman).
- Labels pasted on back of the photographs (no names written)
- Figure legends provided (not more than 40 words)
- Patients' privacy maintained, (if not permission taken)
- Credit note for borrowed figures/tables provided
- Manuscript provided on a CDROM (with double spacing)

Submitting the Manuscript

- Is the journal editor's contact information current?
- Is the cover letter included with the manuscript? Does the letter:

1. Include the author's postal address, e-mail address, telephone number, and fax number for future correspondence?
2. State that the manuscript is original, not previously published, and not under concurrent consideration elsewhere?
3. Inform the journal editor of the existence of any similar published manuscripts written by the author?
4. Mention any supplemental material you are submitting for the online version of your article. Contributors' Form (to be modified as applicable and one signed copy attached with the manuscript)