AUGMENTING NUTRIENT AVAILABILITY OF WHEAT BRAN AND DE-OILED RICE BRAN THROUGH SOLID SUBSTRATE FERMENTATION FOR BROILER CHICKEN

THESIS

Submitted in partial fulfilment of the requirements for the degree
of
Doctor of Philosophy
in
POULTRY SCIENCE

By
Dr. Chintamani Pandurang Munj
Roll No. 1177

To
DEEMED UNIVERSITY
INDIAN VETERINARY RESEARCH INSTITUTE
IZATNAGAR - 243 122 (U.P.)

2011

Dedicated to...

My Beloved Aai, Baba

&

Family members

केन्द्रीय पक्षी अनुसंधान संस्थान,
इज्जतनगर – 243 122

DIVISION OF AVIAN NUTRITION & FEED TECHNOLOGY
CENTRAL AVIAN RESEARCH INSTITUTE
(Deemed University)
IZATNAGAR - 243 122, U.P., INDIA

Dr. Pramod Kumar Tyagi, *Ph.D.*
Senior Scientist

Dated: 30.6.2011

Certificate

Certified that the research work embodied in this thesis entitled ***"Augmenting nutrient availability of wheat bran and de-oiled rice bran through solid substrate fermentation for broiler chicken"*** *submitted by Dr. Chintamani Pandurang Munj, Roll No. 1177, for the award of Doctor of Philosophy Degree in Poultry Science at Indian Veterinary Research Institute, Izatnagar, is the original work carried out by the candidate himself under my supervision and guidance.*

It is further certified that Dr. Chintamani Pandurang Munj, Roll No. 1177 has worked for more than 30 months in the Institute and has put in more than 300 days attendance under me from the date of registration for the Doctor of Philosophy Degree in this Deemed University, as required under the relevant ordinance.

(Pramod Kumar Tyagi)
Chairman
Advisory Committee

Certificate

Certified that the thesis entitled, "**Augmenting nutrient availability of wheat bran and de-oiled rice bran through solid substrate fermentation for broiler chicken**" submitted by Dr. Chintamani Pandurang Munj, Roll No. 777, in partial fulfilment of Doctor of Philosophy degree in Poultry Science at Indian Veterinary Research Institute, Izatnagar, embodies the original work done by the candidate. The candidate has carried out his work sincerely and methodically.

We have gone through the contents of the thesis and are fully satisfied with the work carried out by the candidate, which is being presented by him for the award of Ph.D. Degree of this Institute.

It is further certified that the candidate has completed all the prescribed requirements governing the award of Ph.D. Degree of the Deemed University, Indian Veterinary Research Institute, Izatnagar.

Signature of the External Examiner

Name Dr. V.K. KHATTA

Date : 22.10.2011

(Pramod Kumar Tyagi)
Chairman
Advisory Committee

Date : 22.10.2011

MEMBERS OF STUDENT'S ADVISORY COMMITTEE

Dr. A.K. Shrivastava, Principal Scientist & Head
Division of Avian Nutrition & Feed Technology, CARI, Izatnagar

Dr. Praveen Kumar Tyagi, Principal Scientist
Division of Avian Nutrition & Feed Technology, CARI, Izatnagar

Dr. B.C. Das, Senior Scientist
Division of Physiology and Climatology, IVRI, Izatnagar

Dr. Mukesh Singh, Senior Scientist
Livestock Production and Management Section, IVRI, Izatnagar

Dr. S.K. Mendiratta, Senior Scientist
Division of Livestock Product Technology, IVRI, Izatnagar

Acknowledgements

Happiness lies in the pursuit and not in reaching the goal. Alas! This novel experience is nearing an end and I stand with the Kernel of my Endeavour. While pursuing it many a known and unknown hands pushed me forward, lips put elixir in my heart; learned souls put me on the right path and enlightened me with their knowledge and experience. I shall ever remain grateful indebted to all of them.

I express my profound gratitude and sincere regards to my advisor, **Dr. Pramod K. Tyagi**, Senior Scientist, Avian Nutrition and Feed Technology (AN & FT) division, CARI for his inspiring guidance, I consider myself fortunate and greatly privileged to have worked under his supervision. I thank him for his resolute guidance, constant appreciation, encouragement, supervision, ever helping and friendly attitude during entire course of my research work. Words fail to express my indebtedness and deep sense of gratitude to **Dr. A. K. Shrivastav**, Principal Scientist and Head, AN & FT division, CARI for his valuable support and supervision. With stupendous ecstasy and profundity of complacency, I pronounce an utmost degree of gratitude to my co-guide **Dr. Praveen K. Tyagi**, Principal Scientist, AN & FT division, CARI for his creative suggestions, ingenious advice, sagacious guidance, constructive counseling and moral support as well, for completion of this mammoth task. With ineffable gratitude, I thank my advisory committee members **Dr. B. C. Das, Dr. Mukesh Singh** and **Dr. S. K. Mendiratta**, for their timely advice and constant encouragement.

Words can never express the high regards and love I have towards my **Dr. A. B. Mandal**, Principal Scientist and Head, PGET Section, CARI for his constructive suggestions, constant support and constant encouragement throughout the period of this investigations, the course of this thesis work and preparation of manuscript and helps me a lot in data analysis. It was my proud privilege to associate with him and I would ever like to remain in touch with him in future as well. I also convey my thanks to **Dr. Chandra Deo**, for translating Hindi abstract of my thesis. I am highly thankful to **Dr. R. P. Singh**, Director, CARI and **Dr. H.P. Shrivastava**, Ex-Head PGET for providing the necessary facilities during the course of study, research work successfully and also I am highly oblique to scientist of CARI, **Dr. Ram Singh, Dr. Sirajudeen**, **Dr. A. Pawde, Dr. Bais, Dr. V. K. Saxena, Dr. Deepak Sharma, Dr. S. K. Bhanja, Dr. S. Majumdar, Dr. C.K. Beura** and **Dr. (Mrs) Simi Tomar** for their help providing hatching eggs and encouragements during the period of study. I express my sincere gratitude to **Dr. V. B. Chaturvedi**, Senior Scientist, Energy lab, NRL, AN Division for his valuable support for estimation of GE of research samples. I am profoundly thankful to **Dr. A. S. Yadav**, Senior Scientist, Avian Medicine, CARI for his valuable suggestion during microbiological work. I am extremely thankful to the **Dr. M. C. Sharma**, Director cum Vice

Chancellor, Deemed University, IVRI, and **Dr. V.P. Singh**, Joint Director (Academic), for providing me the financial support in the form IVRI Institute Fellowship, and research grants/facilities for conducting research works as per my objectives during my tenure.

I like to express my sincere appreciation and thankfulness to the technical staff **Mr. Kabadwal, Mr. P. N. Bajpai, Mr. Kundan Kumar** and **Mr. S. P. Singh** for rendered technical cooperation had been of great help. Also, thanks to **Mr. Anwar, Mr. H. C. Singh, Mr. Asgola, Mr. Paul**. My special thanks to dear labmates, **Dr. Shiv Kumar, Mr. Yogesh Kumar, Rampratap, Sapna, Ritu, Radha, Rajni** without whom this work never be materialized. Thanks is due to supporting staffs **Mr. Rawat, Mr. Sunil, Mr. Pappu, Mr. Subash, Mr. Nazir, Mr. Surendra, Mr. Mool Chand, Mr. Ram Pal, Mr. Narayan, Mr. Jiten** and **Mr. Pant** is extremely acknowledged.

I can not forget the company of seniors, classmates and juniors (Drs.) **Kuldeep, Shrikant, Bhupendra, Vinu, Sagar, Tambe, Rahul Patil, Shanker Kumar Singh, Azmat, Shoaib, Santosh, Ajit, Showkat, Satish, Prakash, Sameer, Nilesh, Amar, Krishnna, Sachin, Gajanan, Darya, Abhijit, Rahul, Noni, Hajra, Somen, Mahesh, Ravindra, Suyog, Arjun, Shivaji, Amitkumar, Avinash, Yogya n Yogesh, Ram, Pintya, Vinay, Sangaratna, Mazi, Prarabdha, Suraj, Amol, Prabhakar, Girish, Gautam, Somnath, Dyaneshwar, Praveen, Ramchandra, Abhijeet, Dhanaji and Govind, Prashant, Amit, Anil, Laxmi** *and all* **MAHA mess Boarders**, for their pleasant company, cooperation and valuable help.

"Where the emotions are involved words cease to exist", I owe the depth of gratitude and respect to my beloved **parents, family members, brothers, sisters** and ma ***'pillu'***. Specially, I never forgets sweet voice of little star **Prachi, Pranita, Chetan, Nitu, Sundar, Prasad** and **Sid**. I am thankful to **Mr. Dharmendra (Chachu), Javed Khan** and **Aamil**, for their meticulous typesetting and designing of this manuscript. I express my sympathetic to the experimental broiler chickens who sacrifices themselves for all cause of science and knowledge.

At last, I am extremely thankful to whole **IVRI** and **CARI**, their staff and students for their unforgettable cooperation, innocent love and constant inspirations. I would have never come to this study and engaged myself without their efforts.

Finally, I would like to thank the **ALMIGHTY** for blessings me with enough patience, endurance and strength in accomplishment of the endeavour.

Date: 30.08.2011

Place: Izatnagar

(Chintamani Pandurang Munj)

Abbreviations

%	-	Percentage
@	-	At the rate
°C	-	Degree celsius
µg	-	Micro gram
µl	-	Micro liter
AA	-	Amino acid
ACHO	-	Available carbohydrates
ad lib	-	Ad libtum
ADF	-	Acid detergent fiber
AIA	-	Acid insoluble ash
AME	-	Apparent metabolizable energy
AMEn	-	Apparent metabolizable energy (nitrogen corrected)
AOAC	-	Association of official analytical chemists
APD	-	Apparent protein digestibility
Avl. P	-	Available phosphorus
BV	-	Biological value
BWT	-	Body weight
Ca	-	Calcium
CF	-	Crude fiber
cm	-	Centimeter
CMI	-	Cell mediated immune response
CP	-	Crude protein
CW	-	Citrus waste
D	-	Diet
d. f.	-	Degree of freedom
DCP	-	Di-clacium phosphate
DFRB	-	Defatted rice bran
DM	-	Dry matter
DMM	-	Dry matter metabolizability
DORB	-	Deoiled rice bran
DORP	-	De-oiled rice polish
DRP	-	De-oiled rice polish
E	-	Enzyme(s)
EE	-	Ether extract

ERB	-	Extracted rice bran
ERPM	-	Pearl millet reconstituted with enzymes
ERS	-	Sorghum reconstituted with enzymes
ERW	-	Wheat reconstituted with enzymes
et al.	-	et alli; and others
F	-	Fermentation
FAO	-	Food and Agriculture Organization
FCR	-	Feed conversion ratio
FDA	-	Food and Drug Administration
FDORB	-	Fermented de-oiled rice bran
FFRB	-	Free fatty rice bran
FOS	-	Fructo-oligosaccharides
FPI	-	Foot pad index
FTGM	-	Fermented TGM
FTPase	-	Filter paper degrading activity
FWB	-	Fermented wheat bran
g	-	Gram(s)
GE	-	Gross energy
GEM	-	Gross energy metabolizability
GEM	-	Gross energy metabolizability
GRAS	-	Generally recognized as safe
h	-	Hour (s)
H_2SO_4	-	Sulphuric acid
HA test	-	Haemagglutination test
HA titre	-	Haemagglutination titre
HCl	-	Hydrochloric acid
HI	-	Humoral immune
h	-	Hour(s)
IMT	-	Institute of Microbial Technology
IU	-	International unit
IVPPD	-	*In vitro* pepsin-pancreatin digestibility
kcal	-	Kilo calorie(s)
KCl	-	Potassium chloride
kg	-	Kilogram(s)
KH_2PO_4	-	Potassium dihydrgen phosphate
LMIT	-	Leukocyte migration inhibition test

Log	-	Logarithm
LS	-	Lime stone
LSP	-	Lime stone powder
Lys	-	Lysine
M	-	Mole(s)
ME	-	Metabolizable energy
Met	-	Methionine
mg	-	Milligram
Min.	-	Minute(s)
mIU	-	mole International Unit
ml	-	Milli liter
mm	-	Milli meter
MOS	-	Mannan-oligosaccharides
MSS	-	Mean sum of squares
N	-	Nitrogen
n	-	Number
$Na_2HPO_4.2H_2O$	-	Disodium hydrogen phosphate
NaCl	-	Sodium chloride
NaOH	-	Sodium hydroxide
NDF	-	Neutral detergent fibre
NFE	-	Nitrogen free extract
nm	-	Nanometer
NPP	-	Non-phytate phosphorus
NPU	-	Net protein utilization
NRC	-	National research council
NS	-	Non significant
NSP	-	Non starch polysaccharide(s)
NSS	-	Normal saline solution
P	-	Phosphorus
PBS	-	Phosphate buffer saline
PDA	-	Potato dextrose agar
PER	-	Protein efficiency ratio
PHA-P	-	Phytohaemagglutinin-P
Pi	-	Inorganic phosphate
ppm	-	Parts per millennium
PRP	-	Par-boiled rice polish

RBM	-	Rice bran medium
Ref	-	Reference diet
RP	-	Rice polish
rpm	-	Revolutions per minute
Rs	-	Rupees
RS	-	Reconstituted sorghum
RSM	-	Rapeseed meal
RW	-	Reconstituted wheat
S	-	Sorghum
SBM	-	Soybean meal
SBP	-	Sugar beet pulp
SCP	-	Single cell protein
SE	-	Standard error
SEM	-	Standard error of mean
SPF	-	Specific pathogen free
SRB	-	Supplemented rice bran
SRBC	-	Sheep red blood cell(s)
SSF	-	Solid substrate/state fermentation
TA	-	Total ash
TCA	-	Tri-chloroacetic acid
TGM	-	Toasted guar meal
Thr	-	Threonine
TP	-	Total phosphorus
v/v	-	Volume by volume
w/v	-	Weight by volume
w/w	-	Weight by weight
WB	-	Wheat bran
WHO	-	World health organization
wk	-	Week(s)

List of Tables

List of Figures and Plates

Contents

Introduction...

Introduction

In India, broiler production has increased to 1400 million birds per annum requiring nearly 5 million tonnes of compounded feed annually (Bootwalla, 2005; Manwar and Mandal, 2008). Feeds account over 65% of the total cost of poultry production and thus the major means for manipulating poultry production profitable. The major problem of the feed industry is the scarcity and consistent price hike of the available feed resources. Scope exits to use alternate feed ingredients, which can be suitably incorporated in rations to make poultry production cheaper.

Wheat (*Triticum aestivum*) is the second most important cereal crop in India after rice, grown under diverse agro-climatic condition. India is fourth largest wheat producer in the world. During 2009-2010, estimated wheat production in India was about 80.80 million tonnes according to the Economic Survey (Union budget and Economic Survey, GOI 2010-11). Whole wheat yields about 30% bran. Wheat bran (WB) contains about 12-14% crude protein (CP), 3-4% ether extract (EE), 11-12% crude fiber (CF), 8-9% total ash (TA) and 64-65% nitrogen free extract (NFE), (Bhar and Mandal, 2004). It is bulky in nature and has mild laxative effect. It is palatable and can be fed to pigs, poultry, cattle, sheep and horses. It has a laxative effect partly due to the fiber. Due to high fiber levels and the laxative effect, WB should not be fed to young stock (Jacob, 1991). It also contains sugars

5.4, pentosans 25, Ca 0.1, P 1.0, methionine 0.2, lysine 0.7% and energy 1.858 kcal/kg (Swaminathan and Bhagawan, 1969). It is good source of oligo-unsaturated fatty acids (C18:1, C18:2 and C18:3), and vitamins (thiamine niacin) (Ramakrishna *et al.,* 1982). The maximum recommended level of inclusion of WB in diets for broiler is 10%. Further limitations may be imposed by the bulkiness of the material (Evans, 1985).

Rice (*Oryza sativa*) is the principle cereal food in many part of the world and rice bran is the main by-products available from its milling. During 2009-2010, rice production in India has increased more than four times from 22 to 89.09 million tonnes according to the Economic Survey (Union budget and Economic Survey, GOI 2010-11). During the milling of rough rice, several by-products become available for use as animal feed, such as, polished rice 50-66%; broken rice 1-17%; polishing 2-3%; rice bran 6-8% and hulls, 20% (Juliano, 1985). In India, nearly one million tonnes of rice bran is produced every year. Once oil is extracted from rice bran, the left over residue is called de-oiled rice bran (DORB), defatted rice bran (DFRB) or extracted rice bran (ERB). De-oiling of rice bran improves the protein and mineral content (Warren and Farrell, 1990a). However, the metabolizable energy value is lowered due to decrease in oil content and increase in fiber level (Mandal *et al*., 1985; Ichhponani and Makkar, 1989; Warren and Farrell, 1990c). The nutritive value of DORB in terms of CP content is 11.8 to 21.5%, EE 0.5 to 3.5%, CF 6.0 to 19.7%, NFE 45.9 to 58.2%, TA 7.4 to 18.3%, Ca 0.14 to 0.70% and P 1.02 to 2.90% (Mandal *et al.,* 1985; Eshwariah *et al*., 1986). The metabolizable energy (ME) value of DORB has been reported from 1246 kcal/kg (Bhatia, 1969) to 2191 kcal/kg (Zombade and Ichhponani, 1983). The AMEn of DORB was estimated as 2069

kcal/kg in chickens (Mandal and Pathak, 1996). It should be included in broiler diets at a level between 10 and 20% if strategies are not used to decrease the antinutritive activity (Mandal *et al.*, 1985; Gallinger *et al.*, 2004). Inclusion of rice bran in chicken diets in excess of 20% depressed growth, but higher levels can be tolerated by ducklings (Farrell, 1994).

It is well known that the presence of certain anti-nutritive factor like non-starch polysaccharides (NSP) in most of the brans and cereals drastically affect their proper utilization. The main toxic NSPs of WB are arabinoxylan and β-glucans in aleurone layers and arabinoxylans and cellulase in cell wall of pericarp and testa (Henry, 1985). The arabinoxylans as well as gliadin/glutenin ratio in WB proteins increases the viscosity of the intestinal fluid. However, the anti-nutritional or toxic factors present in rice bran include lipases, trypsin inhibitors, haemagglutinin-lectin and phytates etc. termed as phytotoxicins which get accumulated in bran during polishing of rice, hinder and mask the digestibility and availability of nutrients. Therefore, it is used in livestock or poultry feed as low quality ingredient (Gunanwan and Tangendjaja, 1988; Warren and Farrell, 1990b). Bengtsson *et al.* (1992) found that the type of pentosans might also affect viscosity. However, keeping quality of rice bran is improved drastically on de-oiling.

Among other factors limiting the maximum utilization of rice bran in poultry diets is phytin content. About 82% of the phosphorus in rice bran is in the form of phytic acid or phytate making complex with several others and only 18% of this is available to birds (Tyagi *et al*. 1998). Phytate not only reduces phosphorus availability, but also impair the utilization of other minerals such as Ca, Fe, Zn, Cu and Co (Bhavsar *et al.* 2008) and has a negative effect on protein and

energy utilization, probably due to inhibition of digestive enzymes including pepsin, trypsin and α-amylase (Konietzny *et al*. 2006). Studies indicated that the viscous, water-soluble β-glucans and arabinoxylans were mainly responsible for the anti-nutritive properties of WB and DORB in broilers (Annison *et al.*, 1996).

The anti-nutritive activity of soluble NSP is eliminated effectively by enzyme supplementation and removal of this anti-nutritive factor further increase the energy and nutrient utilization (Wyatt *et al.* 1999). Enzyme may be capable of effectively cleaving various pectic polysaccharides. Enzyme added to poultry diet; especially diets containing cereals and its byproducts such as wheat, wheat bran, de-oiled rice bran, barley and rye, not only enhance the nutrient availability of these diets but also produced many other benefits (Bengtsson *et al.*, 1992; Elangovan *et al.*, 2002). *Aspergillus niger* (*A. niger*) is considered as generally recognized as safe (GRAS) microorganism as per WHO and FAO. It is a filamentous fungus and most preferred organism in the industrial production of fermented foods, organic acids and enzymes. The use of filamentous fungi for the production of commercially important fermented products has increased rapidly over the past half century. It has been used in the fermentation industries in manufacturing citrate, oxaloacetate and pyruvate. It also liberates certain enzymes like phytase, glucoamylase, cellulase and pectinase during fermenting process.

Solid-substrate fermentation (SSF) systems have generated much interest in recent years because of several economical and practical advantages. SSF systems have also been reported to be an effective way to produce enzymes. Many strains such as Aspergillus, Fusarium, Chaetomium, Rhizopus, and Trichoderma are being used on commercial scale for this purpose (Solomon *et al.*, 1999). SSF processes

can be defined as "the growth of microorganisms (mainly fungi) on moist solid materials in the absence of free-flowing water" (Moo-Young *et al*., 1983; Pandey, 1992). SSF of bran can increase the nutrients availability and improve their sensory characteristics, thus, adding value to these materials and creating new opportunities for their utilization. Fermentative process, especially SSF had been used to produce value added products from raw materials (Pandey and Soccol, 1998; Anupama and Ravindra, 2001; Laufenberg *et al.*, 2003; Kang *et al.*, 2004).

Keeping in view the above facts, it is necessary to augment availability of feed energy and nutrients, which not only help in saving feed but also help to reduce environmental pollution and bad aroma in poultry house. Therefore, the agriculture products and by-products should undergo some processing methodologies viz., SSF in order to make them suitable for poultry. Scanty information is available in literature regarding fermentation of WB and DORB with *A. niger* and optimum condition for its better growth on wheat and rice bran. Fermented WB and DORB showed the inconsistent results regarding the change in its chemical constituents due to fermentation. Apparently, least work on feeding value of WB and DORB is confined to chicken and only some reports on its use in broiler feeding are available. Scanty literature is available regarding feeding of fungal fermented WB and DORB in broiler and layer ration. Hence, an attempt was made in the present study to examine the potential of *A. niger* for the production of enriched protein on WB and DORB substrates, and their successive use in poultry feed formulations. In view of above facts, the proposed study was planned with the following objectives:

- To optimize fermentation conditions for optimum growth of *A. niger* on wheat bran (WB) and de oiled rice bran (DORB) as substrates.
- To ascertain availability of energy and certain nutrients (protein, carbohydrates and phosphorus) from fermented WB and DORB.
- To evaluate feeding value of fermented WB and DORB on growth, nutrient utilization, carcass traits, immune-responsiveness, gut microbes and certain blood biochemical parameters in broiler chickens.

❖❖❖

Review of Literature...

Review of Literature

The continuous rising cost of feed and feed ingredients is a matter of great concern to all those associated with poultry production. Feed processing includes different kind of treatments applied for maximum utilization of nutrients for poultry. Some of them are simple and some require expensive mechanization. There has been increased interest in developing technologies for low-cost feed processing to improve nutrient utilization. A method of feed processing is selected based on nature of feed, its availability, its chemical composition, and presence of toxic factors, economic implications, the quantity to be processed and use of processed feed ingredients. Feed enzyme supplementation has also been one of the biological means for improved feed utilization. There are reports in literature indicating that the processing improves starch availability and protein utilization resulting in increased feed utilization efficiency.

Wheat bran (WB) and Deoiled rice bran (DORB) are agro-industrial residues that are good sources of proteins, lipids, vitamins and minerals. As they are rich in indigestible carbohydrates which decrease their biological value and make sensory characteristics less acceptable. Both WB and DORB have bulky and palatable nature. WB constitutes about 30% of the whole wheat whereas rice bran takes about 10% by weight of the brown rice. The term bran is usually used in trade to describe a mixture of several tissues. Solid-state

fermentation of bran can increase the nutrients availability and improve their sensory characteristics, thus, adding value to these materials and creating new opportunities for their utilization for broiler chickens. There appears to be paucity of information on the feeding of wheat bran and de-oiled rice bran in broiler chickens. Therefore, pertinent information on this aspect has been briefly reviewed from avian species.

2.1 CHEMICAL COMPOSITION OF WB AND DORB

Saima and Hasmi (1999) reported that WB contains crude protein (CP) 12.23, crude fiber (CF) 6.25, ether extract (EE) 4.86, total ash (TA) 9.56 and nitrogen free extract (NFE) 60%. According to Butt *et al.* (1998), WB contains moisture 9.67, CP 15.75, EE 3.77, CF 10.38 and TA 4.18% on DM basis. Chemical composition of representative WB samples had 18.39 CP, 6.84 CF, 3.39 EE and 5.50% TA on DM basis (Wan *et al.,* 2009). It may contain up to 89% DM, 15.7% CP, 3% EE but can contain up to 12% CF which limit it's use to less than 5% as a feed ingredient in broiler rations (NRC, 1994).

The composition of DORB has been reported to vary quite considerably. Much of the variability in the nutrient composition has been attributed to the variety of paddy rice from which the DORB originated (Soaad *et al.,* 1981), the efficiency of dehusking prior to milling (Zombade *et al.,* 1977), the method of milling itself and the presence of adulterants such as rice hulls or husk of little nutritional value (Houston, 1972; Warren and Farrell, 1990a).

The comparative proximate composition of WB and DORB has been shown by Verma (1995) as 13.0 and 12.0% CP, 2.50 and 15.0% EE, 64.5 and 45.0% NFE, 7.0 and 15.0% TA, 0.10 and 0.08% calcium (Ca) content and 1.0 and 1.5% phosphorous (P) content but according

to Sen *et al.* (1978) 12.8 and 10.4% CP, 11.1 and 22.6% of CF, 64.3 and 38.1% NFE, 32 and 7.5% EE, 8.4 and 21.5% of TA content. It is also shown by Singh (1997) as 92.07 and 84.46% DM, 17.35 and 16.65% CP, 17.89 and 26.08% CF, 2.95 and 0.81% EE, 7.93 and 15.54% TA, 0.95 and 5.74% acid insoluble ash (AIA), 0.81 and 0.74% Ca content and 1.12 and 1.21% P content.

Carbohydrates form an important part of the WB and DORB, and which have been fractionated according to their bio-availability. WB and DORB have been reported to contain 300 and 350 g/kg DM cellulose, 500 and 250 g/ kg DM hemicellulose and 150 and 170 g/ kg DM lignin, respectively (Couto and Sanroman, 2006). The high content of hemicelluloses (around 14%) as reported by Warren and Farrell (1990a) is, however, an important factor to be looked into carefully for the chicken, have a limited ability to utilize dietary hemicelluloses such as that from barley or from guar, the latter usually exerts negatively on the utilization of dietary nutrients (Verma and McNab, 1985). It makes an attractive feedstock for conversion to a variety of value-added products such as single cell protein (Ravinder *et al.*, 2003, 2006).

WB is also known for its powerful phytase activity (Eeckhout and De paepe, 1993). Nearly 20% of WB-phytate could be hydrolysed through water treatment which is a relatively simple, easy to apply and an inexpensive process (Tyagi *et al.*, 1998b). The bran's phytase may improve the absorption of phosphorus from cereals and their bran when given to simple stomach animals (Lesson and Summers, 2008). WB and DORB both provide more phosphorus than calcium, which influences the dietary mineral balance with high level of supplementation (White, 1965; Ahmed *et al.*, 1989) and for this reason limestone additives are often recommended.

2.2 AMINO ACID COMPOSITION OF WHEAT BRAN AND DE-OILED RICE BRAN

Almost similar amino acid contents in the WB and DORB has been reported by Almquist (1948) that in same order are 0.90 and 0.82 % arginine, 0.56 and 0.52% lysine and 0.35 and 0.35% threonine on DM basis. Bran has amino acid balance superior to that of whole wheat (Cheeke, 1991). The detailed comparative data of amino acid contents in WB and DORB is also shown in Table 1 (Wahal and Saxena, 1979).

Table 2.2.1 Amino acid composition of WB and DORB

Amino acids	WB/100 g Protein	DORB/100 g Protein	WB/100 g DM	DORB/100 g DM
Tryptophan	1.91	0.77	0.34	0.11
Lysine	3.76	3.85	0.67	0.55
Histidine	1.91	1.54	0.34	0.22
Arginine	6.29	3.85	1.12	0.55
Hydroxyl proline	Nil	1.24	Nil	0.18
Aspartic acid	8.40	8.64	1.50	1.24
Threonine	2.55	3.08	0.45	0.44
Serine	5.67	5.83	1.01	0.83
Glutamic acid	19.72	20.34	3.51	2.91
Proline	6.19	5.76	1.10	0.82
Glycine	5.67	10.11	1.01	1.45
Alanine	5.97	3.71	1.06	0.53
Cystine	1.91	0.77	0.34	0.11
Valine	4.44	4.62	0.79	0.66
Methionine	0.62	2.25	0.11	0.32
Isoleucine	3.76	3.08	0.67	0.44
Leucine	5.67	4.62	1.01	0.66
Tyrosine	2.53	7.87	0.45	1.13
Phenylalanine	3.15	3.08	0.56	0.44

2.3 METABOLIZABLE ENERGY VALUE OF WHEAT BRAN AND DE-OILED RICE BRAN AS PREDICTED FROM NUTRIENT COMPOSITION

2.3.1 Metabolizable energy value of WB

Reddy and Vaidya (1973) reported 1069 kcal/ kg DM AMEn value for WB whereas, Mandal (1992) reported the AMEn value of WB 1020 kcal/ kg and 1007 kcal/ kg on DM basis for adult male chicken and guinea fowl, respectively. Cilliers *et al.* (1999) reported somewhat higher ME content of WB at 2845 kcal ME/ kg. On the other hand, some researchers reported somewhat low ME value of WB which was in range of 1110 - 1300 kcal/kg (Miles and Nelson, 1974; Scott, 1982 and NRC, 1994). Cilliers *et al*. (1999) evaluated the effect of the enzymatic hydrolysis of phytate on available energy content of WB for chicks and the ME value was 1660 kcal/ kg for treated WB. Positive impact of enzyme supplementation was not evident. This was presumably due to the increased heat production in these birds caused by improved nutrient absorption and utilization.

2.3.2 Metabolizable energy value of DORB

AMEn value of DORB has been found to be 2937 kcal/kg DM (Reddy and Vaidya, 1973), whereas Mandal, (1992) reported the AMEn value of DORB at 2069 kcal/kg and 1998 kcal/ kg on DM basis for chicken and guinea fowl, respectively. Wide variability observed in the ME value have been attributed to the dietary inclusion level of the test material (Bhatia, 1969; Mandal *et al.*, 1974), age, sex and strains of the birds (Pym and Farrell, 1977), level of dietary fiber (Lodhi *et al.*, 1976) and chemical composition of the diet. The ME value of DORB for chickens has been reported by several workers as shown in Table 2.

Table 2.3.1 Metabolizable energy value of DORB (Kcal/kg DM)

Type of bird	Type of diet used	Inclusion level (%) 20	40	Source
Chicks	Practical	1246	2235	Bhatia (1969)
Chicks	Practical	1717	2077	Bakshi (1971)
Broiler chicks	Practical	2191	-	Zombade & Ichhponani (1983)
Cockerels	Practical	2317**	-	Eshwaraiah *et al.* (1986)
Cockerels	Practical	1000-2000*	-	Ichhponani & Makkar (1989)
Chickens	Practical	1750	-	Warren & Farrell (1990)
Cockerels	Practical	2240	-	
Chicks	Practical	1557	1601	Verma *et al.* (1990-91)
Quails	Batch type	1408	1718	Bhanja (1992)
	Continuous type	1545	2053	

*Calculated value ** True metabolizable energy value

2.4 ANTI NUTRITIONAL FACTORS PRESENT IN WB AND DORB

The nutrive value of bran, as of any other product, is not only the result of its total nutrient content but depends also on nutrient availability and digestibility. The use of bran in poultry nutrition is limited by its low digestibility. The cellulose-hemicellulose matrix of the aleurone cell walls acts as a barrier to the attack on nutrients by avian digestive enzymes. Moreover antinutritive factors, originally present in the cereal grain, limit the availability of bran nutrients or act as enzyme inhibitors (Di Lena *et al.,* 1997). The main toxic non-starch polysaccharides (NSPs) of WB are arabinoxylan and β-glucans in aleurone layers and arabinoxylans and cellulase in cell wall of pericarp and testa (Henry, 1985). Studies indicated that the viscous, water-soluble β-glucans and arabinoxylans were mainly responsible for the anti-nutritive properties of WB in broilers (Annison *et al.,* 1996). However, the anti-nutritional or toxic factors present in rice bran include lipases, trypsin inhibitors, haemagglutinin-lectin and

phytates etc. termed as phytotoxicins which get accumulated in bran during polishing of rice, hinder and mask the digestibility and availability of nutrients. Therefore, it is used in livestock or poultry feed as low quality ingredient (Gunawan and Tangendjaja, 1988; Warren and Farrell, 1990d). The pancreatic hypertrophy induced by trypsin inhibitor has been reported in poultry and rats when fed raw rice bran (Barber *et al.,* 1978; Ikegami *et al.,* 1990). Phytic acid as phytates inhibits the digestibility and availability of nutrients in rice bran by formation of complexes with minerals, protein, digestive enzymes and amino acids particularly lysine, methionine, arginine and histidine (Reddy *et al.*, 1982).

2.5 WHEAT BRAN, DE-OILED RICE BRAN AND INTESTINAL VISCOSITY

The young chicks are unable to utilize WB and DORB effectively, attributed to the consequence of digestive inadequacy due to increased viscosity of gut contents. Viscosity seems to mediate not only an impaired nutrient digestibility, but also modification in morphology and histology of the intestine, protein and energy metabolism and microbial population in the gastro-intestinal tract (Bedford *et al.,* 1991). Enzyme supplementation significantly improved diet AME content whereas viscosity of ileal contents was reduced significantly (Preston *et al.,* 2001). Partial and complete hydrolysis of NSP indirectly reflects the viscosity of intestinal digesta.

2.6 FEEDING VALUE OF WB FOR POULTRY

WB is a by-product of flour industry that is obtained from screened grains of wheat, which has a limited use for human, and monogastric animals due to its high fiber content. It is well known that broilers cannot properly utilize fibrous materials because their

inability to digest cellulose cell wall. It is reported that wheat bran may contain up to 12% crude fiber (NRC, 1994). One of the ways of using feeds that are under normal circumstances denigrated is by use of fermentation techniques (Dirar, 1992). Abasiekong (1991) observed an improvement in the feeding value of spent sorghum when fermented with a stock culture of some rumen microorganism and reported direct fermentation of spent sorghum with rumen fluid produced similar results that could be utilized on farm. On the other hand, Aduku (1993) reported that wheat offal contains 1256 and 2320 kcal of ME per kg for poultry and swine, respectively, 15.6% crude protein and mineral elements such as calcium and phosphorus. Dale (1996) suggested that the ME value of wheat by-products was directly proportional to their fiber content and that ME can be described as in the following formula: 3182 – 161 x %CF (kcal/kg). Yao *et al.* (2007) reported that nutritive value of the analyzed WB was as follows: DM 88.57%, CP 15.52%, total phosphorus (TP) 0.89, Ca 0.13, TA 4.70, CF 8.34% and phytase activity 2400 U/kg. Because WB has some phytase activity, it can be used as a viable source of phytases. Zanini and Sazzad (1999) reported that phytase is an enzyme that breaks down the indigestible phytic acid (phytate) portion in grains and releasing digestible phosphorus and calcium for nonruminants.

Viveros *et al.* (2000) studied the phytase and acid phosphatase activities in plant feedstuffs. In this study, 24 feedstuffs were analyzed for total phosphorus, phytate phosphorus content, phytase, and acid phosphatase activities with the objective to predict the capacity to hydrolyzed phytic acid and to contribute to formulating environmentally adequate diets for monogastric animals. These authors reported that approximately two-thirds of phosphorus in plants is in the form of phytate and concluded that wheat bran contains

4624 U/kg phytase and 14106 U/kg acid phosphatase. Pallauf *et al.* (1994) reported that phytate phosphorus is unavailable to or poorly utilized by poultry due to the very low phytase activity found in their digestive tract. Therefore phytase is added to poultry diets to improve the utilization of phytate phosphorus.

Eeckhout and De Paepe (1994) reported that some feedstuffs contain 6-phytase activities (i.e. wheat, WB, rye and barley), whereas other feedstuffs have little or no phytase activity (i.e. corn, oat, sorghum and oilseeds). Barrier-Guillot *et al.* (1996) reported that the phytase activity in grain such as wheat has a high correlation with overall phosphorus retention in pig and broiler (r=0.83). Cavalcanti and Behenke (2004) studied the effect of wheat bran phytase subjected to different conditioning temperatures on phosphorus utilization by broiler chicks based on body weight and toe ash measurement. These authors reported that WB has high endogenous phytase enzyme activity and concluded that phytases can improve the plant phosphorus digestion by the broiler chicks. Also they revealed that wheat bran phytase resulted in an increase in growth rate and phosphorus utilization in broiler. Earlier studies have suggested that high endogenous phytase in cereals and their by-products can effectively enhance phosphorus utilization by monogastric species (Pointillart, 1991). WB has been reported to have phytase activitity at 2957 ± 1556 U/kg, it ranged from 1180 to 5208 U/kg from sample to sample (Eeckhout and De Paepe, 1993). Nevertheless, Steiner *et al.* (2007) found that WB contains 6-phytase activities ranging between 2349 and 9945 U/kg. Paik (2003) reported that the presence of 6- phytases in WB is high enough to be considered in feed formulation for monogastric animals.

Tyagi *et al.* (1998b) found the effect of water soaking of WB on phytate phosphorus autolysis and its feeding value to chicks. Soaking WB in water (1:1, w/v) for 6 or 12 hr at room temperature (23-27°C) did not change in proximate composition but for phytate P which was hydrolysed into inorganic (available) P to the extent 7.31 and 19.51%, respectively. Feeding to chicks was found beneficial in that body weight gains and feed consumption increased significantly and little improvement in feed efficiency.

Previous research showed that phytase activities were lowest in legume seeds and oats (262-496 U/kg), and highest in cereal by-products such as WB (2957-9945 U/kg) (Eeckhout and De Paepe, 1993), and differences in the phytase activity of cereals and their by-products may come from cultivars, processing and measurement methods (Steiner *et al.* 2007).

Yao *et al.* (2007) studied the effects of wheat bran phytase on performance and nutrient utilization of laying hens and concluded that wheat bran phytase improved the performance and utilization of total phosphorus and crude protein of laying hens, and reported that ten percent of WB replacing 0.05% inorganic phosphate (Pi) did not influence either egg yield or nutrient utilization. This study suggests that WB could be used successfully in laying hen diets and wheat bran and microbial phytase supplemented together could replace inorganic phosphate completely. Further, WB could be an economical source of protein especially in developing countries; though its high fiber content limits its use as a feed ingredient in poultry rations, especially those of broilers.

Patil (1972) reported the utilization of WB in day-old chickens, given rations alone or with 10 to 70% WB in place of maize and SBM.

There was steady fall in body weight as WB increased in the diets. Body weights were significantly depressed with 40% WB or more; with 70% WB they were half that of controls. In another trial, rations containing 0 to 60% WB, 21.7 to 14.9% protein and a constant calorie: protein ratio of 64.0 was used and body weights were reduced by group receiving 20% WB which was the first significant reduction and 50 and 60% inclusion of WB, reduction in body weights was about half that of control weights. In third experiment, rations containing 50% WB and calorie: protein ratio 41.3, 46.8, 52.5, 58.5 or 64.7 were used. Body weights were increased in calorie: protein ratios up to 58.5; all groups given WB were significantly lighter than controls.

Layer diets containing wheat bran (WB) at 30% were compared to a maize/groundnut cake. Feed intake was significantly enhanced while weight gain was not affected. Layers on WB, and CD had similar egg production rate and feed per kg egg (Odunsi *et al.*, 2002).

Recent studies (Abaza *et al.*, 2004; Ali *et al.*, 2006a&b) demonstrated that WB alone or WB supplemented with some enzyme preparations have a positive effect on the performance of broilers and laying hens. Ali *et al.* (2006a,b) studied the effects of WB using up to 50% in the layer ration and concluded that the detrimental effect of inclusion of WB at higher rate can be overcome by addition of sodium sulfate or enzymes. Christopher *et al,* (2007) studied the effect of replacing maize with wheat offal in broiler finisher diets on bird performance and feed cost. These authors found that replacing maize with about 25% wheat offal in the broiler finisher diet has no adverse effects on growth, feed intake and efficiency of feed utilization; however, feed cost was reduced considerably.

On the other hand WB contains a large amount of betaines, which protects chick intestinal cells from coccidian infection, alleviates symptoms and improves performance (Kettunen *et al*, 2001). Zeisel *et al.* (2003) reported that wheat bran contains betaine at a rate of 1505.6 mg/100g.

2.7 FEEDING VALUE OF DORB FOR POULTRY

Icchponani and Makkar (1989) reported reduced energy content of DORB due to extraction of oil. Higher CF content of DORB exerts an adverse effect on the utilization of energy and protein in poultry. In fact, a significant negative correlation has been established between the CF and energy content and with protein digestibility of the ration. Bakshi (1971) reported that de-oiled rice polish (DORP) could be successfully used up to 30% level in diet of growing chicks. Protein and nitrogen utilization from diets containing rice polish (RP) and DORP up to 30% level were similar. Mandal *et al.* (1974) reported that DORP can be safely included at least up to 20% level in the diet of chicks without adversely affecting growth and feed efficiency. In fact, the growth was slightly improved by incorporation of DORP than the respective reference ration.

Zombade and Ichhponani (1983) determined the nutritive value of raw, parboiled, stabilized and defatted rice bran for growing chicks. These inclusions depressed body weight gains of broiler chicks by 183, 79, 49 and 200g when levels of respective bran were raised from 200 to 400 g/kg in the respective diets. However, the feed and protein conversions were significantly depressed at both levels of rice bran in the diets.

Verma and Shrivastava (1989-90) conducted an experiment to investigate the response of White Leghorn hens to dietary DORB vis-

à-vis normal RB on their productive performance. Results suggested that inclusion of DORB up to 30% level in isonitrogenous diets resulted in slight decrease in dietary energy level and significantly lower egg production in birds fed such a diet. However, it had been concluded that DORB can well be used for RB in the diet of hens up to 20% level without seriously affecting the egg quality although the egg performance and efficiency of feed utilization slightly decreased.

Warren and Farrell (1990c) reported that when de-fatted rice bran with a high fiber and lower ME content than free fatty rice bran (FFRB) replaced up to 21% of a basal component of chicken starter diet, good growth comparable to the control birds was obtained. Later in another study, Warren and Farrell (1991) examined the apparent retention of minerals and digestibility of amino acid from diets based on RB in male growing chickens and adult cockerels. The apparent retention of Ca declined with increasing bran inclusion and in many instances the values were negative. Retention values for Mg and P measured with chickens and adult cockerels were positive. Although these did not change substantially with increased RB inclusion in the diets, the values were usually lower on diets with than without RB.

Deolankar and Singh (1979) showed a depression in Ca and Fe availability to chickens of mixed diets containing RB relative to a maize-based control. They assumed that reduction in both Ca and Fe availability was the result of the phytate content of RB and postulated the formation of calcium-fatty acid soap in the intestine which may have reduced the absorption of Ca. Nwokolo *et al.* (1977) examined the effects of both phytate and CF on the retention of minerals in the chick using four byproduct meals. They also found significant inverse relationship between the availability of most of

the minerals and CF and phytate levels. Zinc was least available but in general mineral availability was over 50%. They felt the effect of fiber may have been more important than phytate. Warren and Farrell (1991) also reported lower retention of Ca and P with the higher phytate level.

The physical and chemical composition of fiber varies from bran to the other. Wheat bran contains higher hemicelluloses which is hydrophilic in nature. Its water holding capacity and bulk density are also higher, resulting in laxative nature. Contrary to this, the hemicelluloses content of rice bran is much lower than that of wheat bran (Singh, 1997).

2.8 NUTRITION FOR OPTIMIZING THE IMMUNE RESPONSE

The problem of immunosuppression has been felt to be prominent due to various factors *viz*. managemental conditions, nutritional status, intensive production system, high-density rearing and infectious diseases. Therefore, it is highly essential to find ways and means for enhancement of immune response by nutritional manipulation. With high-density confinement of poultry under commercial environment, important role of nutrition is that the birds should not only fed for production or reproductive performances but also must be fed to minimize infectious diseases and their concomitant stresses. Substantial information is available in literature to indicate that administration of certain vitamins, minerals, amino acids and their different combinations to chicken in excess of their supposed requirements enhance their disease resistance. This increased resistance attributed to significant stimulation of humoral and cellular immunity and phagocytosis. Since, the use of antibiotics has been limited, better use of supplementary

immuno-stimulatory nutrients has to be made in poultry feeding. Immunity to an antigen that causes the production of antibodies indicated in the very young chick. Two immune systems develop in the body of a chick, carried away by two types of lymphocytes. In a young chick, certain lymphocytes originating in the yolk sac and bone marrow pass through the thymus are known as T lymphocytes. After maturation, they congregate in lymphoid organs like spleen, caecal tonsils, payer's patches etc. T-cells do not produce antibodies but can destroy foreign cells by direct contact, which is referred as cell-mediated immunity. Some of the immature lymphocytes pass through bursa of Fabricius where they mature and later congregate in lymphoid organs. Specialized cells known as plasma cells in the B system including bursa, spleen etc. are responsible for the production of antibodies. In later stages of life B- lymphocytes are constantly produced by lymphoid organs and no longer require bursa for maturation.

Immunity is usually classified as innate (natural) or acquired (specific). Innate immunity includes physical/chemical barriers, phagocytes, neutrophils, the complement system, and natural killer cells, and macrophage derived cytokines such as a- and b-interferon and tumor necrosis factor (Abbas *et al.*, 1991). Acquired immunity, induced by natural exposure to antigen or vaccination, includes lymphocytes, antibodies (immuno-globulins) and lymphocyte derived cytokines such as interlukin-2, interlukin-4, and transforming growth factor-b (Abbas *et al.*, 1991). Acquired immunity is further divided into either humoral or cell-mediated immunity. Humoral immunity is mediated by B-lymphocytes and involves interaction of various cell types and culminates in antibody formation. Humoral immunity provide defense against extra cellular microbial infections. In cell-

mediated immunity, mainly the T-lymphocytes and lymphocyte-derived cytokines provide defense against intracellular pathogens and tumor cells. Cell mediated immune response include lymphocyte transformation, cell mediated cytotoxicity, delayed type hypersensitivity, transplant rejection and lymphokine secretion.

Nutrition is an important modulator of immune function and can often influence the balance between health and disease. Optimizing the immune system is important because responses with the wrong leukocyte populations or under-responsiveness can increase the incidence of infectious diseases. Poor immunocompetence can result in greater incidence and duration of infections, which cause decreased feed intake, nutrient losses, and impaired animal health and well-being. Feed influence immunity through several mechanisms, which include nutrient needs of immune system cells, through direct regulatory effects on immune system, and physical and chemical immunomodulation action of non-nutrient components of feeds. Energy, amino acids and all nutrients are necessary for the anabolic activity of immune cells (leukocytes), such as proliferation and antibody production as well as the secretion by the liver of large quantities of immunologically active molecules, the acute phase proteins. In young animals, a severe deficiency of virtually any nutrient impairs immunocompetence (Cook, 1991).

Nutrients in the diet can directly affect the regulatory functions of leukocytes altering the type, duration, and vigor of the immune response. Feeding regimes markedly affect insulin, glucagon and glucocorticoid, levels, which can change the type and duration of the immune response. When broiler chickens are restrictively fed, insulin levels are decreased and glucagon levels are increased.

Changes in these hormone levels affect the chicken's ability to mobilize neutrophils, which affects their resistance to various types of diseases. Other dietary factors that influence immunity through their effects on hormone levels include protein to calorie ratios and presenting feed *ad lib*. versus in a few large daily meals. Very little organized research has been conducted concerning the effects of energy intake on immune responsiveness.

Investigation on the effect of feeding diets containing various levels of WB and DORB with or without enzymes on the cell mediated immune (CMI) response of broilers appears to be scanty in literature.

Gujral (2005) studied the effect of fructo-oligosaccharides (FOS) and mannan-oligosaccharides (MOS) on immune response in growing quails. The antibody titers of the chicks on various dietary treatments on first, second and third week post immunization were found non-significant between MOS or FOS and also no significant difference due to interaction of these oligosaccharides. Similar findings were observed by Zhou *et al.* (2005) in specific pathogen free (SPF)chicken. However, the CMI response as shown by Leukocyte migration inhibition test (LMIT) was significantly increased with supplementation of MOS (Gujral, 2005).

Manwar (2007) studied the effect of addition of feed enzyme, reconstitution on utilization of various cereals, optimum production and immune competence in broiler chickens. When the types of wheat compared, the birds diets containing wheat reconstituted with or without enzymes showed increased ($P>0.01$) immune response measured in terms of humoral response to SRBC compared to their counterparts fed raw or supplemented with enzymes. Compared to control group, there were no statistical significant differences in CMI response in all the groups.

Bhutia (2006) observed that the titer values were tended to increase at 14th days of immunization and did not differ significantly among different dietary group on the basis of inclusion of toasted guar meal (TGM) and enzyme on humoral immune (HI) response. Dinani (2009) studied the effect of SSF of TGM and its comparison with or without enzyme supplementation in broiler quail ration. And result was reported that HI response was significantly higher in fermented TGM (FTGM) group as compared to other dietary groups. However, both the researchers conducted the LMIT in broiler quails and observed an increased titer values at 14th day post-immunization and did not differ significantly among dietary groups on the basis of inclusion levels of TGM, FTGM and enzyme.

2.9 GUT MICROBIAL POPULATION

The small intestine of the chicken is usually colonized by facultative anaerobes whereas the caeca are dominated by strict anaerobes. Utilization of nutrients through microbial conversion of digestible carbohydrates, such as starch, to volatile fatty acids is less efficient than direct absorption of glucose released from enzymatic digestion. Increased amount of soluble NSP in the diet may lead to development of an undesirable gut micro flora and fermentation. Chickens fed with fructo-oligosaccharides had a four-fold reduction in the level of salmonella present in the caeca (Bailey *et al.*, 1991). However, Annison *et al.* (1996) concluded that rice polishing NSP may have been a substrate for hindgut fermentation in the broiler. The possible reason for the reduction in the colonization of total microbial load may be due to the presence of highly viscous polysaccharides in de-oiled rice bran which acts as a substrate for microbes and might have increased microbial fermentation in caeca. Dietary oligosaccharides attract microbes away from intestinal binding

site and therefore reduce colonization of pathogenic microbes (Lee *et al.* 2003a). Choct *et al.* (1995) observed that enzyme supplemented eliminates the increased fermentation in small intestine which caused due to presence of large amount of viscous NSP in wheat based diet.

Vahjen *et al.* (1998) studied the influence of xylanase supplementation to wheat based diet on the development of selected bacterial group on the intestinal tract of broilers and observed that the less viscous intestinal environment caused by the xylanase addition slowed proliferation of gram-positive cocci and presumptive enterobacteria enzyme–supplemented birds. Enzyme supplementation also exhibited significant reduction in microbial count of caecal contents (Bhutia, 2006; Dinani, 2009).

2.10 NUTRITIONAL IMPROVEMENT THROUGH FEED PROCESSING

It is well known that the presence of anti nutritive factor i.e. certain NSP in most of the cereals and bran such as wheat bran and de-oiled rice bran etc., used as feed ingredient drastically affect their utilization. Enormous efforts have been made by nutritionist to improve NSP digestion in the birds by using various methods. Water treatment, heat treatment, autoclave, antibiotic supplementation, enzyme supplementation and solid substrate fermentation are the several ways to alleviate the deleterious factors in the cereals and bran. The improvement of the nutritive value of barley, corn and wheat by a simple water treatment has been reported five decade ago (Fry *et al.*, 1958). Water treatment probably removes the water soluble NSP (pentosans and β-glucan) and activates endogenous enzymes capable of degrading these NSP. The degree of improvement is obviously dependent on the concentration of water soluble NSP in the cereals and its bran.

Osborne and Mendel (1917) for the first time observed the effect of heat treatment in improving nutritive value of soybean. Heat ruptures the enzyme-resistant carbohydrate-protein bonds and makes the nutrients available for poultry. Maillard reaction and other deleterious reaction may result due to heat processing of the grains or bran and it may lead to creation of new linkages between peptide chains (Carpenter, 1973). Heat treatment causes denaturation of proteins and gelatinization of starch resulting into improved ME (Hill and Renner, 1963). Heat treatment of grains also increased amino acid availability (Ingram *et al.*, 1949; Reisen *et al.*, 1997). Soybean meal cooked at 112-130°C contains protein with twice the nutritive value of raw soybeans. Hot water soaking or tannin extraction of sorghum improved FCR in broilers (Musharaf and Latshah, 1991). The optimum temperature and pressure for heat treatment of proteins reported to be 110°C for 30 min at 1.345 kg/cm^2 atmospheric pressure (Parsons, 1943). Heating of grains or bran also eliminates the heat labile anti-nutritional factors such as trypsin inhibitors, haemagglutinins and saponins in soybean and gossypol in cotton seed meal. Heat treatment of grains or bran increases their water absorption ability, facilitates faster conversion of starch to soluble carbohydrates, sugars and energy. The improved nutritive value due to grinding attributed to cell wall breakage and release of nutrients to the digestive enzymes and mechanical release of intracellular starch. Cabrera (1994) reported improved nutritional value when particle size was 100 to 500 microns and best performance with diet in medium particle size (1.13-1.23 mm) than 0.57 to 0.67 mm. Grinding of feed ingredients may result in reduced gizzard weights, gizzard pH, and pH of intestinal contents and rate of passage of feed is inversely proportional to particle size with which affects nutrient

absorption (Hamilton and Proudfoot, 1995). It has been reported that energy requirement of birds depends upon the form of the diet. However, Zhuge *et al.* (1990) found no effect of particle size on chick performance. Reece *et al.* (1985) reported that roller mill required less energy for grinding. Hamilton and Kennie (1997) observed improved body weight gain and feed intake in broiler turkeys given mash than those fed pellets. Popping is a method of dry hot processing of grains and it is accomplished by raising the temperature of the grains to 178°C without moisture. It involves heating of grains at an air temperature of 370-425°C. In treated grains, 3% moisture is lost and causes expansion of grains. The density of the grains is reduced and it improves digestibility of starch.

Micronization involves popping of grain with the application of infrared heat. Microwaves with $3x10^8$ to $3x10^{11}$ cycles per second are used. The temperature of the grain increased to 149°C. Starch granules of the micronized grains were completely gelatinized and extensively swelled. Processing cost is extremely low. Igbasan and Guenter (1996) reported improved true ME and average amino acid availability and decreased lysine availability in adult cocks and further they reported increased egg production, FCR and egg mass in laying hens fed micronized peas. Douglas (1991) reported that micronization of high tannin sorghum and maize resulted into improved starch availability as well as weight gain and FCR in broilers. Domacinovic *et al.* (1999) observed that chickens fed micronized cereals grew faster, reduced FCR and showed smaller proportion of abdominal fat. Further, they reported that micronized cereals in chicken feeding are justifiable considering the production results and cost of processing. Pelleting is a mechanical process of densification of a ground grain or composite feed with or without

application of moisture or steam. The optimum temperature for pelleting of poultry diet is 65-70°C. Increased temperature and time of heating reduces lysine availability in maize. Effects of pelleting vary with method of preparation and feedstuff combination. The use of pellets as a mean of improving poultry feed dates back 1930. By 1950, pellet feeding became more acceptable because of the phenomenal growth of broiler industry and use of relatively bulkier material in feed formulae. The formation of diets into pellets or crumbles has become a routine practice and pelleting of poultry rations improves diet density from 0.57 to 0.71. Broilers should be fed high-density diet as broilers fed less dense diet grew slower (Hussar and Robble, 1962). Reddy *et al.* (1961) reported that feed consumed as pellets have 30% more productive energy than that consumed as mash. The rule of thumb is that the pelleting of the feed will produce 2 pounds more broiler meat per 100 pounds of feed and will allow the grower to market birds of a given weight two days earlier. Scheele (1996) suggested that pelleted diets, which stimulate feed intake and protein accretion, would increase the incidence of ascites in vulnerable birds.

Dry roasting refers to treatment of grains with dry heat, resulting in expansion in volume of grains and increased digestibility. The moisture content of the grains was reduced by 5%. Gupta *et al.* (1995) also observed improved broiler performance. Latshaw (1974) reported higher egg production with higher roasting temperature of soybeans. During exploiding, the grain first subjected to steam at high pressure in a closed chamber and suddenly decreased to atmospheric pressure resulting into rapid expansion of the grain. Generally, steam pressure of 15.9 to 17.6 kg/cm^2 for 15-20 seconds is used. Extrusion is a special method of cooking. Extruding machine

applies heat and pressure by means of friction. Mercier and Feillet (1975) found maximum expansion at 170-200°C with improved α-amylase digestibility of raw starch from 18 to 80%. Improved weight gain and FCR in broilers fed extruded rice bran also reported in literature (Sayre *et al.*, 1988).

2.11 APPLICATION OF ENZYMES IN FEED PROCESSING

Enzymes are highly efficient catalyst from biological sources and catalyses all synthetic and degradable reactions in the body. Search of novel microbes resulted in to production of thermostable, acidophilic or alkalophilic enzymes operational under different conditions. Enzymes are used in diets with wheat and barley for a number of years in eastern countries. There has been interest in the use of enzymes to enhance the performance of poultry on maize-soybean based diets. Scanty information is available on the use of feed grade mixed enzymes in wheat bran or de-oiled rice bran-based diets. Supplementation of feed grade mixed enzymes improved growth and feed conversion efficiency partly in pearl millet-based diets during early age of broiler chickens but had no beneficial effect on maize-soya based diets (Mandal *et al.*, 2002). Added enzymes can enhance the nutritional value of poultry diets. The effect on chick performance depends on the type of antinutritive factor present in the diet, the nature of other ingredients, the type and amount of enzymes used, and the age of the animal. The nutritional value of cereal byproducts viz. wheat bran and de-oiled rice bran that contain a high level of viscous water-soluble NSPs can usually be improved to a marked degree by the addition of the appropriate enzyme to the diet. In general, barley and oats have a high content of soluble b-glucans and therefore, respond to β-glucanase, whereas wheat and rye tend to have higher contents of arabinoxylans and therefore respond to

xylanases and possibly other associated enzymes. Maize, in contrast, contains very low levels of water soluble NSP. Therefore, nutrient utilization is not depressed, and as a result, maize does not show a response to enzyme treatment. Enzyme addition to a diet high in water soluble NSP has been shown to improve weight gain, FCR, apparent protein digestibility, apparent lipid digestibility, and the AME of the diet. In addition, enzymes reduce the size of the gastrointestinal tract, the water content of excreta, and the incidence of vent pasting. When properly used, enzymes can produce many beneficial effects in the chicken and other kinds of poultry. Most of the enzymes currently used in the food and beverage industry are from *Aspergillus*, but hemicellulases and cellulases are derived from *Trichoderma*. Recently, genes encoding for different enzymes, including phytases, b-glucanases, and xylanases, have been cloned and expressed in different commercial systems (microorganisms and plants).

Enzymes that have been used over the past several years or have potential for use in the feed industry include cellulase, xylanase and associated enzymes such as phytase, protease, lipase and galactosidase. Enzymes in the feed industry have mostly been used for poultry and, to a lesser degree, for pigs to neutralize the effects of the viscous NSPs in cereals such as barley, wheat, rye, and triticale. These anti-nutritive carbohydrates are undesirable, as they reduce digestion and absorption of all nutrients in the diet, especially fat and protein.

Reduction in gut viscosity, particularly in jejunum by supplemental enzymes was observed in broiler fed diets based on wheat (Veldman and Vahl, 1994; Choct *et al.*, 1995; Vander Klis *et al.*, 1995ab; Steenfeldt *et al.*, 1998). As a consequence of enzyme

supplementation, reduced viscosity enhanced the passage rate of digesta, increased feed intake and performance. Dusel *et al.* (1998) found that addition of xylanase enzyme to wheat-based diet reduced digesta viscosity in both intestinal segment (jejunum and ileum).

Recently, considerable interest has been shown in the use of phytase as a feed additive, as it not only increases the availability of phosphorus in plants but also reduces environmental pollution. Several other enzyme products are currently being evaluated in the feed industry, including protease to enhance protein digestion, lipases to enhance lipid digestion, b-galactosidases to neutralize certain antinutritive factors in non-cereal feedstuffs, and amylase to assist in the digestion of starch in early-weaned animals. Studies indicated that the viscous, water-soluble β-glucans and arabinoxylans were mainly responsible for the antinutritive properties of wheat, wheat bran, rye, triticale, barley, and oats in broilers (Annison and Choct, 1991; Bedford, 1995; Choct *et al.*, 1996). The degree of improvement obtained by adding enzymes to the diet depends on many factors including the type and amount of bran in the diet; the level and type of antinutritive factor in the bran, the spectrum and concentration of enzymes used and the age of the birds. A decrease in size of intestine following enzyme addition is presumably related to a more efficient and rapid digestion of nutrients, reducing the need for an enlarged intestine. Reduced water intake and water content of the excreta are probably related to a reduced ability of the partially hydrolyzed water soluble NSP to absorb water. The latter effect is of considerable importance, as a wet excreta is associated with vent pasting, soiled birds and eggs. These observations indicate that enzyme supplementation of cereal-based diets or cereal byproduct-based diets can have a dramatic effect on the performance and

probably also the physiological responses of chickens. There appears to be a relationship between the growth-depressing effect of the cereals or cereals byproducts and their content of viscous NSP. Bengtsson *et al.* (1992) found that the type of pentosans might also affect viscosity. Enzymes added to poultry diets; especially diets containing cereals such as wheat, barley, and rye, not only enhance the nutrient availability of these diets but also produce many other benefits.

Enzyme supplementation significantly improved diet AME content whereas the viscosity of ileal contents was reduced significantly (Preston *et al.*, 2001). Choct and Annison (1990) reported that wheat pentosans decreased protein utilization by increasing the endogenous amino acid secretion as well as by inhibiting the true digestibility. Added pentosans in wheat-based diet reduced digestibility by 20% (Fengler *et al.*, 1988). A negative effect due to its soluble fiber has been documented but this could be alleviated by addition of fiber degrading enzymes. Digestibility studies with wheat usually revealed a starch digestibility between 0.93 and 0.98 (Choct *et al.*, 1999; Annison, 1990).

McCracken and Quintin (2000) reported that enzyme addition improved gain and FCR but had no effect of wheat or enzyme addition on AME contents of the diet. Svihus and Heltland (2001) indicated that an overload of wheat starch in the digestive tract might be the cause of poor digestibility in broilers. However, Sikka and Chawla (2002) found no improvement in weight gain and but improved feed utilization efficiency due to low feed consumption. Wyatt *et al.* (1999) reported that removal of anti nutritive effects of soluble NSP increased the nutrient utilization and the energy content. Further, they reported that the diets supplemented with enzyme increased energy

availability by 3.3%. Supplemented enzyme did not improve the growth rate of broilers fed wheat based diets but reduced the feed consumption resulting into improved protein and energy utilization in the broilers (Preston *et al.*, 2001).

Hammond (1994) concluded that addition of proteinase from fungal or plant origin inactivated rice bran lipases without denaturing the natural proteins. The effect of enzyme supplementation on dry matter digestibility in poultry depends on the type of diet and type of animal. The increase in dry matter digestibility ranged from 0.90 (Schutte *et al.*, 1995) to 1.7% (Annison and Choct, 1993) in poultry. Similarly, Classen (1996) also reported enzyme hydrolysis activities are most effective in improving starch utilization. However, presence of anti-nutritive factors in cereals and digestive capacity of chick might affect the utilization of complex carbohydrate i.e. arabinoxylan.

Farrell and Martin (1998) determined strategies to improve rice bran by testing the efficacy of 2 enzyme preparations targeted the NSPs in rice bran. In their three experiments RB (400 g/kg) decreased chick and duck performance. There was a decline in growth rate and feed intake with increasing inclusion of RB at a level of 0, 200, 400 g/kg. It was concluded that NSPs were not a significant factor in suppressing the nutritional value of rice bran and therefore, the uses of enzyme preparations were unlikely to be beneficial.

2.12 APPLICATION OF SOLID SUBSTRATE FERMENTATION

The process of solid substrate fermentation (SSF) initially concentrated on enzyme production. But presently, there is worldwide interest for single cell protein (SCP) production due to the dwindling conventional feed resources (Zadrazil and Puniya, 1995; Nigam and Singh, 1996).

SSF causes changes in food quality indices including texture, flavour, appearance, nutrition and safety. The benefits of fermentation may include improvement in palatability and acceptability by developing improved flavours and textures, preservation through formation of acids, alcohol, and antibacterial compounds, improved digestibility of protein and carbohydrates, removal of antinutrients, natural toxicants and mycotoxins. Fermentation also improved the quality of cereal proteins (Chavan *et al.*, 1988). Natural fermentation of cereals increased their relative nutritive value and available lysine content (Hamad and Fields, 1979). The bacterial fermentations involving proteolytic activity expected to increase the biological availability of essential amino acids more than yeast fermentations, which mainly degrade carbohydrates (Chavan and Kadam, 1989). Starch and fiber tended to decrease during fermentation of cereals (El-Tinay *et al.*, 1979). Fermentation did not alter the mineral content of the product. However, the hydrolysis of chelating agents such as phytic acid during fermentation improved the bioavailability of minerals. However, changes in the vitamin content of cereals with fermentation varied with the fermentation process, and the raw material used in the fermentation and the group vitamins showed an increase on fermentation (Chavan and Kadam, 1989). During the fermentation of maize, thiamine levels were virtually unchanged, but riboflavin and niacin contents were almost double (Steinkraus, 1994). Reddy and Pierson (1994) reviewed the effect of fermentation on anti-nutritional and toxic components in plant foods. Fermentation of maize meal and soybean-maize meal blends lowered flatulence factor carbohydrates, trypsin inhibitor and phytates (Chompreeda and Fields, 1981; 1984). However, fermentation of cereals with fungi, such as *Rhizopus oligosporus*, reported to release bound trypsin

inhibitor, thus increasing its activity (Wang *et al.*, 1972). Fungal and lactic acid fermentations have been reported to reduce aflatoxin B1, sometimes by opening of the lactone ring, which resulted in complete detoxification (Nout, 1994).

The highest content of crude protein was obtained with SSF treatment. It was found that there was a synergistic effect on crude protein production, fiber degradation and the secretion of enzyme system (Ke *et al.*, 2011). Some results showed that the protein production and the strong degradation of fiber after solid state fermentation (SSF) of the substrate by the fungus *A. niger* which enables it to be used as a feed supplement in diets for poultry, and represents a useful destination for the bran and other agro-industrial residues.

Toasted guar meal fermented with *A. niger* and *Fusarium sp.* fed to chicks showed higher growth rate, FCR and better utilization of DM and protein as compared to toasted and autoclaved guar meal (Nagra, 1984). Verma and McNab (1985) reported that incubation of guar meal for 60 h at 30°C with *A. niger* increased CP from 37 % to 42%, phosphorus content from 0.65 to 0.98 % due to release of fungal phytases. SSF of guar meal with *A. niger* markedly enhanced its feeding value to chicks with significant decrease in viscosity of the guar meal.

Reddy and Rao Eshwaraiah (1986) studied the effect of autoclaving and solid substrate fermentation of raw, de-oiled rice polish (DRP) and par-boiled rice polish (PRP) in broiler diets and reported that autoclaved or fermented rice polishing replaced 1/3rd or 2/3rd maize in control diet. The weight gains or feed efficiency in chicks fed autoclaved rice polishing were comparable to control diet. No specific trend in weights of liver and spleen were noticed

with inclusion of rice polishing. It was observed that autoclaved rice polishing caused a lower pancreatic weight. They suggested that the factor causing pancreatic hypertrophy in raw rice polishing was heat labile.

Khan *et al.* (1990) produced biomass containing 24.80% protein by *Trichoderma harzianum* from defatted rice polishing. The biomass was biologically evaluated through a 15 days feeding trial on broiler chick by replacing 25% and 50% of vegetable protein results obtained were in terms of apparent protein digestibility (APD) 70.95, 77.89 and 77.74, net protein utilization (NPU) 24.13, 23.50 and 22.81 and biological value (BV) 22.46, 30.96 and 29.42% respectively for the three rations (without BP, with 25% and 50% replacement). Bajwa *et al.*, (1991) obtained biomass containing 15.17% protein when 0.5% alkali treated rice straw was fermented with *Arachinotus sp.* with $CaCl_2$ 0.01, $MgSO_4.7H_2O$ 0.1, KH_2PO_4 0.2 and cane molasses 1.0 % with C: N ratio 25:1 at pH 4.0 and 40^0C after 6 days of incubation.

Mahmood *et al.* (1991) investigated the effect of different micronutrients on the production of single cell protein by *Arachinotus sp.* and a biomass produced contained 17.23% CP by fermenting rice polishing in the presence of $CaCl_2$ 0.01; $MgSO_4.7H_2O$ 0.1 and KH_2PO_4 0.2 at pH 4 and 30^0C temperature.

Akhtar *et al.* (1997) obtained SCP containing 15.31 and 10.93% CP containing 6.75 and 3.28% true protein from wheat bran and grain bran with *Arachniotus sp.* after 6 days of incubation. In case of biomass produced from wheat bran and grain bran, methionine was the first limiting amino acid and chemical scores were 56.41 and 20.82 respectively. The second limiting amino acids were lysine and isoleucine, respectively.

Shojaosadati *et al.* (1999) studied to enrich the protein content of sugar beet pulp (SBP), wheat bran (WB) and citrus waste (CW) by solid state fermentation (SSF) using *Neurospora sitophila.* Optimum conditions for protein enrichment of these lignocellulosic byproducts by *N. sitophila* under SSF were investigated. Optimal moisture content for growth on SBP and CW was 75–80% (w/w) whereas for WB it was 65% (w/w). The protein content of untreated SBP, WB and CW after 5 days SSF on surface was increased from 15, 13 and 7% (w/w) to 30, 30 and 18.2%, respectively.

Anupama and Ravindra (2001) were made attempt to apply the SSF for the production of Single Cell Protein (SCP) using oil free RB as substrate. Carbonate-bicarbonate extraction buffer and a pH 10 were found to be most efficient among the buffers used for the extraction of the proteins. The effect of supplementation by various sources of nitrogen and mineral solution on the final biomass yield was compared. The influence of C/N ratio on the protein yield was also studied. Sodium nitrate at C/N ratio of 1.387 was found to be an effective nitrogen supplementing source, as it gave higher biomass yield. Rice bran was best substrate to yield total protein content.

Rudravaram *et al.* (2006) studied the optimization of protein enrichment of DORB by SSF using *A. oryzae* MTCC 1846. The optimum conditions for the enrichment process were found to be moisture content 60%; temperature 28^0C; pH 6.0; inoculum's concentration 109 spores/g substrate and particle size of DORB, 0.3 mm. Among the various nitrogen source tested, ammonium sulfate (0.6% w/w) showed maximum protein enrichment (24.30%) followed by vegetable + fruit waste extract (23.50%) and legume root extract (23.10%).

Shojaosadati *et al.* (1999) attempted to enrich the protein content of lignocellulosic substrates by solid state fermentation using

Neurospora sitophila. Also, optimum conditions for protein enrichment of these lignocellulosic byproducts were investigated. They found that optimal moisture content for growth on sugar beet pulp (SBP) and citrus waste (CW) was 75–80% (w/w) whereas for WB it was 65% (w/w). The protein content of untreated SBP, WB and CW after 5 days SSF on surface was increased from 15, 13 and 7% (w/w) to 30, 30 and 18.2%, respectively.

Saima and Hasmi (1999) studied the suitability of WB as a substrate for the production of biomass protein and to assess its nutritive value through chemical analysis and biological trial on broiler chicks. And they found that biomass protein of WB increased CP from 12.23 to 35.67%, and decreased NFE 60.00 to 14.65%, thus biomass has the potential to replace 50% of the fishmeal because of its high protein content and good nutritive value. Simillar, optimum condition were reported by Bashir *et al.* (1990) for the production of biomass from the wheat bran using *C. utilis* as a fermentative organism. He obtained the maximum biomass protein in shacked medium. *C. utilis* has also been used for fermentation of rice polishing with maximum crude protein (27.8%) at 9 % substrate level after 72 hrs of incubation at pH 4 and 30^0C (Kiani, 1989).

Oshoma and Ikenebomeh (2005) studied the growth of *A. niger* on rice bran medium in submerged fermentation by using standard methods for determinations of dry biomass, total crude protein and final pH values. The total protein on dry weight basis was 28 % for rice bran medium (RBM). However, the total protein percentage on dry weight basis was 32% crude protein content for supplemented rice bran (SRB) medium, 33% for GRB medium and 28% for RBM. Other workers, Ravinder *et al.* (2003) recorded 43% protein content

when *A. oryzae* was cultivated in de-oiled rice bran. Protein content of 18-25% was observed when *Penicillin javanicum* was grown on rice husk (Khan *et al.*, 1990). In essence, rice bran was used as a potential source for product with higher protein content by utilizing cellulose present in the rice bran. Overall results indicated that *A. niger* can be used as a potential strain for SCP production. Since rice bran was successfully utilized for the enrichment of protein in product, there is a possibility of converting agroresidue waste to proteinaceous feed and food.

Pogaku *et al.* (2009) studied the effect of the protein enrichment by *A. oryzae* MTCC 1846 on DORB, it was found to be 13.20% followed by 12.90% and 12.70% for *A. niger* MTCC 1842 with and *T. viride* NRRL 1186 respectively, for single cell protein production using fungal cultures under solid state fermentation. They reported that protein enrichment of the selected fungi on DORB after 60 h of incubation. All the three fungi increased protein content of DORB, but differ in amount of protein enrichment. The protein enrichment of *A. oryzae* MTCC 1846 on DORB was found to be 13.20% followed by 12.90% and 12.70% for *A. niger* MTCC 1842 with and *T. viride* NRRL 1186 respectively. Among the three fungi *A. oryzae* MTCC 1846 shows minimum content of nucleic acids (5.3%) while *A. niger* MTCC 1842 and *T. viride* NRRL 1186 showed (6.1%) and (7.2%), respectively.

Dinani (2009) studied solid substrate fermentation of toasted guar meal (TGM) and its comparision with or without enzyme supplementation in broiler quail ration. Fungal fermentation of TGM increased CP from 41.48 to 59.28%, total phosphorus from 0.42 to 0.53% and decreased urease activity from 0.023 to 0.011 mg nitrogen/g/minute at 30^0C and NFE (mostly NSP gums) 39.67 to 19.51%, thus enhancing the nutritional worth of TGM.

Saima *et al*. (2007) studied the production and biological evaluation of microbial biomass by the fermentation of melon-peels with *A. niger* and introduced a new protein source for poultry birds and reduced pollution problems caused by peels. In phase-I, they revealed that fungal fermentation of melon peels increased CP from 14.22 to 35%. Then various conditions like substrate: water ratio, carbon: nitrogen ratio, temperature, addition of molasses, pH and incubation period were optimized for fermentation *by A. niger*. Results revealed that maximum CP contents were observed at 6 g substrate, 7:1 carbon to nitrogen ratio, 25^0C, 1% molasses, 4.5 pH and total incubation time was 72 hours for fermentation. In phase-II, a ten days trial was conducted to evaluate protein quality of biomass in terms of protein efficiency ratio (PER) and Net protein utilization (NPU) which were 35.71 and 50.41, respectively.

Darwazeh (2010) investigated the fermentation of wheat bran with rumen filtrate. The crude protein content in wheat bran increased from 13.6% to 14.2%, GE from 3905.4 kcal/g to 3599 kcal/g when fermented with rumen filtrate. And the bran's fiber content decreased from 12 to 9.5%, TA content from 4.0 to 3.9% when the wheat bran is fermented for the specified period of time. And investigated the effect fermented wheat bran with rumen liquor at different inclusion rates on the performance of broilers at age from 21-35 days. A total of 205 one-day-old male and female *Cobb* broiler chicks were fed commercial diets from 1-20 days of age. The chicks were fed the experimental diets from 21-35 days of age. Feed consumption, weight gain, feed conversion ratio and carcass characteristics were not significantly affected across treatments. The results of this study indicated that fermented wheat bran with rumen filtrate up 15% inclusion rate can be used without any adverse effects in the broiler finisher diet.

The contribution of specific enzymes to indigenous cereal byproduct fermentations is perhaps less known than that of microorganisms. Further research should be directed towards identifying the benefits and risk associated with specific indigenous fermented cereal byproducts; elucidating the contributions of microorganisms, enzymes and other cereal constituents in the fermentation process; developing unique microbial strains and technique for nutritive improvement and detoxification, and testing of new cereal byproducts.

2.13 PHOSPHORUS UTILIZATION

In general, phytic acid is relatively heat stable and short time cooking does not significantly affect the phytate content of legumes and cereals. In seed, phytase activity increases during germination to release phosphorus for the developing plant (Chang, 1967). Phytates are present in most cereals but their activity varies widely among cereals (Bartinik and Szafranska, 1987). It is generally accepted that phytase can completely hydrolyze phytic acid to the monophosphate form and in some cases to free innositol or orthophosphate. Takemasa and Murakmi (1995) explored the possibility of reduction of phosphorus excretion of chicks by use of barley or wheat. The non-phytate phosphorus (NPP) of the diet can be decrease from 0.40 to 0.32 percent by use of barley or wheat at the 50 per cent lend in the diet resulting in a 20% reduction of the phosphorus excretion of chicks. A promising means to increase the efficiency of phosphorus use in poultry production is to add phytase to poultry diets. Simons *et al.* (1990) observed that phytase supplementation of a low phosphorus maize-soybean diet increase the availability of phosphorus to over 60% and decreased the amount of phosphorus in the droppings by 50%. Recent advances in enzymology and fermentation technology have resulted in large-scale commercial

production of feed grade phytase. Ravindran *et al.* (1999) found phytase effective in enhancing utilization of phytate in wheat soybean based diets in broiler feeds. Shah *et al.* (2009) used various carriers for application of phytase in feed. WB and DORB were superior to silica and calcium carbonate. Effect of phytase on release of phosphorus from agriculture residues was studied and revealed that initial phosphorus in WB and DORB was 4.02 mg and 4.0 mg. Due to submerged fermentation, released (after phytase action) to 7.01 mg and 7.2 mg, respectively. Several studies indicated that microbial phytase supplementation increased the availability of phytate phosphorus in broiler chickens fed maize-soya based diet (Simons *et al.*, 1990).

Tyagi *et al.* (1998b) studied the effect of water soaking of WB on phytate phosphorus autolysis and its feeding value to chicks. Soaking WB in water (1:1, w/v) for 6 or 12 h at room temperature (23-27°C) did not change in proximate composition but for phytate P which was hydrolysed into inorganic (available) P to the extent 7.31 and 19.51%, respectively. And inferred that feeding water treated WB to growing chicks improved their performance through improved phosphorus utilization. Birds fed FWB had 7.9% phosphorus per gram ash compared to 6.7% for the birds receiving the control diet (Darwazeh, 2010). These results were similar with those of Cavalcanti and Behenke (2004) who reported that wheat bran phytase resulted in higher growth rate and higher phosphorus utilization by broiler. Earlier studies (Pointillar, 1991) suggested that endogenous phytase of cereal and cereal by-products enhance phosphorus utilization by monogastric species. We believe that further studies need to be conducted to study the effects of FWB inclusion at graded levels in broiler diets at the same time when phosphorus content of the diet is held constant.

❖❖❖

Materials and Methods...

Materials and Methods

The present investigation was carried out to study the effect of solid substrate fermentation of wheat bran and de- oiled rice bran with or without enzymes on nutrient availability, growth performance and immune response in broiler chickens. The experiments were conducted at the Division of Avian Nutrition and Feed Technology, Central Avian Research Institute, Izatnagar. The experimental procedures and analytical techniques followed are detailed as under.

3.1 EXPERIMENT 1: OPTIMIZING MOISTURE LEVEL FOR GROWTH OF *A. NIGER* ON WHEAT BRAN AND DE-OILED RICE BRAN SUBSTRATES

3.1.1 Procurement and maintenance of culture

The pure culture of *A. niger* (MTCC ACC No. 281) was procured from the Institute of Microbial Technology (IMT), Chandigarh, India. The lyophilized culture was reconstituted and subculture was made by agar plating technique using potato dextrose agar (PDA). The inoculated subculture was kept at 37^{o}C for 48 h and washed with 15 ml of 0.01% Tween - 80. Autoclaved water (15 lbs for 15 min.) was used for spore suspension and further dilution. Spore suspension was prepared at 1 lac spore per ml concentration. *A. niger* spores were harvested by tapping. Spore count was determined by using a haemocytometer according to the Fuchs-Rosenthal technique (Guillard, 1973). The spores were preserved at 4^{o}C until the organisms were used for solid medium inoculation.

Calculation of Fuchs-Rosenthal:

$$\text{No. of cells per ml} = (n_1 + n_2) / 2 \times 10^3 \times D$$

Where, n_1 = number of cells counted in upper rafter
n_2 = number of cells counted in lower rafter
D = dilution factor

3.1.2 OPTIMIZATION OF CONDITION FOR *A. NIGER* GROWTH ON WHEAT BRAN AND DE-OILED RICE BRAN

In- vitro trials were conducted in order to optimize the conditions for growth of *A. niger* on WB and DORB. The WB as well as DORB were treated separately in laboratory scale by maintaining different levels of moisture at constant temperature (37^0C) and duration (72 h).

1. Moisture level 1: WB or DORB and water to soak at the ratio of 70 and 30 (w/v).
2. Moisture level 2: WB or DORB and water at the ratio 60 and 40 (w/v).
3. Moisture level 3: WB or DORB and water at the ratio 50 and 50 (w/v).

Optimum pH (4.5 to 6.5) required for the growth of *A. niger* fungus was maintained by addition of dilute acid (HCl) in WB and DORB soaked in water for autoclaving. Optimum moisture concentration for growth of *A. niger* growth was judged by visual inspection and chemical analysis. The most suitable moisture level was assessed through dry matter loss and chemical analyses (AOAC, 2000).

3.2 EXPERIMENT 2: EFFECT OF SOLID SUBSTRATE FERMENTATION OF WHEAT BRAN AND DE-OILED RICE BRAN ON NUTRIENT AVAILABILITY

3.2.1 Solid substrate fermentation (SSF) of WB and DORB with *A. niger*

After optimization of conditions, the fermented wheat bran (FWB) and fermented de-oiled rice bran (FDORB) were prepared for

use in the experimental diets. WB and DORB were soaked in tap water and mixed thoroughly. It was autoclaved at 15 psi for 15 min. Then, it was spread uniformly in a tray of 1.5 to 2.0 cm thick layer. Spore suspension was inoculated at the rate of one lac spore per kg of autoclaved WB and DORB. The polythene sheet (300 gauge) was spread over the tray after inoculation and spraying of spore suspension. The polythene sheet was folded on all the sides of the tray to create anaerobic condition for fermentation in BOD incubator. The best moisture level standardized in the earlier experiment was maintained for final fermentation. Subsequently, the bran were forced air oven-dried at 60°C for 3 d. The fermented bran was then (with or without enzyme) tried on day-old broiler chicks up to their marketable age to find out their feeding value and improvement in feeding value, if any. They were compared simultaneously with the untreated bran at the same levels.

3.2.2 Chemical analysis

Six samples of fungal fermented WB and DORB obtained in triplicate by different levels of moisture combination and one each representative sample of WB and DORB were analyzed for their nutrient composition (CP, CF and EE) following standard techniques. Also, phosphorus (AOAC, 1990) and calcium (Talpatra *et al.*, 1940) contents of the fermented bran were analyzed. Gross energy of all that samples was determined by using Ballistic Bomb Calorimeter. Screening for aflatoxins (AOAC, 1990) and urease activity (GAFTA, 1995) was done for all the samples of fermented WB and DORB as well as WB and DORB as such. Beside this, all processed samples and representative sample of WB and DORB were evaluated *in-vitro* for the following parameters:

A metabolism trial was conducted involving two substitution level (20 and 40%) of reference diet with raw or fermented wheat bran and de-oiled rice bran in cockerels following practical diet replacement method (Sibbald and Slinger, 1963). Each diet was replicated six times having one cockerel per replicate.

EXPERIMENTAL PLAN

The experiment was conducted in a well-ventilated open shed with ceiling fans. The birds received natural light for about 12 h, however, no artificial light was provided. Each cockerel was kept individually in raised wire floor metabolism/layer cage (replicate), with separate feeding and excreta collection facility. The dimensions of the cages used for rearing cockerels were 45 cm long x 30 cm wide x 46 cm high. During the preliminary feeding period of 10 days, the birds were fed on conventional grower diets. Thereafter, the reference diet and the test diets (Table 3.2.1 and 3.2.2) at two substitution level (20 and 40%) were offered to six replicated groups for a 12 days adaptation period. The birds were given free access to water and experimental diets.

The feed intake during adaptation period was recorded to ascertain the intake capacity of birds. This was followed by a collection period of three days. During this period, a weighed quantity of feed was offered to match the previous mean feed intake of the birds. Precautions were taken to avoid spillage of feed using properly designed feeders measuring 16 cm long x 9.5 cm wide x 13.5 cm in deep for cockerels. The total excreta collection method was employed. The excreta samples were dried at 60^{o}C in a hot-air oven. The dried feed, residue if any and excreta samples were ground and assayed for gross energy using a Gallenkamp Ballistic Bomb Calorimeter

(Model No. CB 370, *Expotech*. USA. *Inc.*, Houston, Texas). The nitrogen contents of the feed, residue and excreta samples were determined (AOAC, 1990). The dry matter and energy metabolisability of cockerels for four different samples of bran (raw and fermented) at two substitution levels of bran were calculated.

Table 3.2.1: Ingredient and nutrient composition (%) of diets used for ME bioassay of WB and FWB

Ingredient	Ref. diet	Test diet							
		T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8
Maize	78.00	63.94	63.94	63.94	63.94	36.00	36.00	36.00	36.00
SBM	22.00	16.06	16.06	16.06	16.06	24.00	24.00	24.00	24.00
WB (Raw)	-	20.00	-	-	-	40.00	-	-	-
FWB (70:30)	-	-	20.00	-	-	-	40.00	-	-
FWB (60:40)	-	-	-	20.00	-	-	-	40.00	-
FWB (50:50)	-	-	-	-	20.00	-	-	-	40.00
Mineral-vitamin premix*	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Total	101	101	101	101	101	101	101	101	101
Nutrient composition									
CP[1]	15.17	15.66	14.60	14.24	14.88	19.67	19.64	18.51	19.32
GE, kcal/g[1]	4.284	4.640	4.320	4.305	4.433	4.128	3.886	4.256	4.093
ME, kcal/kg[2]	3103	2752	2781	2781	2781	2336	2336	2336	2336

Mineral and vitamin premix @ 1kg /100 kg over and above to supply iodized salt 200 g, calcium carbonate 200 g, dicalcium phosphate 500g, manganese 4 g, zinc 3.4 g, iron 2.3 g, copper 0.26g, retinol 248 mg, cholecalciferol 0.3 mg, riboflavin 555 mg, thiamin 70mg, pyridoxine 70 mg, niacin 280mg, calcium pantothenate 35 mg and cyanacobalamin 70 mcg.

WB-Wheat bran, FWB- Fermented wheat bran

[1]Analyzed values, [2]Calculated value

Calculation of AMEn

The AMEn values of the diets were calculated by the method of Hill and Anderson (1958). The AMEn values of the diets were then multiplied by 1.01 since the mineral and vitamin premix were superimposed @ 1 kg /100 kg diet. The AMEn values of the test

ingredients were calculated as AMEn of test diets after correction for minerals and vitamin supplement = 0.80 R + 0.20 T or = 0.60 R + 0.40 T (where R is the AMEn of the reference diet after correction for mineral and vitamin supplement and T is the AMEn of test ingredient). The classical and nitrogen-corrected AME values of test diets at both substitution rates. The data were analysed statistically (Snedecor and Cochran, 1969) for dry matter and energy metabolisability in a 4 x 2 factorial completely randomised design with four bran (1 raw + 3 substrate: moisture level) and two substitution rates (200 and 400 g/kg) of bran.

Table 3.2.2: Ingredient and nutrient composition (%) of diets used for ME bioassay of DORB and FDORB

Ingredient	Ref. diet	Test diet							
		T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8
Maize	78.00	63.94	63.94	63.94	63.94	36.00	36.00	36.00	36.00
SBM	22.00	16.06	16.06	16.06	16.06	24.00	24.00	24.00	24.00
DORB (Raw)	-	20.00	-	-	-	40.00	-	-	-
FDORB (70:30)	-	-	20.00	-	-	-	40.00	-	-
FDORB (60:40)	-	-	-	20.00	-	-	-	40.00	-
FDORB (50:50)	-	-	-	-	20.00	-	-	-	40.00
Mineral-vitamin premix*	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Total	101	101	101	101	101	101	101	101	101
Nutrient composition									
CP[1]	15.17	15.52	14.58	15.62	15.73	19.57	19.59	19.91	19.73
GE, kcal/g[1]	4.284	4.225	4.589	4.327	4.311	4.078	3.987	4.122	4.277
ME, kcal/kg[2]	3103	2892	3038	3038	3038	2557	2612	2612	2612

Mineral and vitamin premix @ 1kg /100 kg over and above to supply iodized salt 200 g, calcium carbonate 200 g, dicalcium phosphate 500g, manganese 4 g, zinc 3.4 g, iron 2.3 g, copper 0.26g, retinol 248 mg, cholecalciferol 0.3 mg, riboflavin 555 mg, thiamin 70mg, pyridoxine 70 mg, niacin 280mg, calcium pantothenate 35 mg and cyanacobalamin 70 mcg.

DORB-De-oiled rice bran, FDORB- Fermented de-oiled rice bran

[1]Analyzed values, [2]Calculated value

3.2.4 *In vitro* pepsin-pancreatin digestibility (IVPPD)

In vitro pepsin-pancreatin digestibility of raw and processed bran samples was measured according to the method of Gopalkrishnan and Prakash (2000). For the determination of IVPPD, 1 g of finely ground bran was heated over a water bath with 5 ml of water for 10 min., suspended in 15 ml of 0.1 N HCl, containing 1.5 mg pepsin in a 100 ml conical flask. The mixture was incubated at 37°C for 3 h. The suspension was then neutralized with 0.5 N NaOH and treated with 4 mg pancreatin in 7.5 ml of 0.2 M phosphate buffer (pH 8.0), containing 0.005 M sodium azide. The mixture was incubated for different time intervals (30-50 min.). Ten milliliters of 10% tri-chloroacetic acid (TCA) was added to the mixture to stop the reaction. The mixture was then centrifuged at 3000 rpm for 20 min. at 27°C. Nitrogen in the supernatant was estimated by micro-Kjeldahl method. A blank was also prepared in the same manner without the sample and the value was subtracted from total digestibility of each sample (Akeson and Stahmann, 1964).

3.2.5 Determination of available carbohydrates

The available carbohydrate contents (ACHO) of raw and processed brans were determined by the method of Clegg (1956). The method in brief was as follows.

Preparation of reagents

Anthrone reagent

A quantity of 60 ml of concentrated H_2SO_4 added to 330 ml of water with stirring and allowed to cool. One gram each of thiourea and anthrone were added and stirred until dissolved and stored in refrigerator.

Glucose stock solution

An amount of 400 mg of anhydrous glucose in water dissolved and diluted to 500 ml. i. e. 0.8 mg/ml (prepared just before use).

Working standards

For preparation of working standards, 5, 10, 15, 20 and 25 ml of stock solution was taken in 100 ml volumetric flask and diluted to the mark to achieve required concentration of 0.04, 0.08, 0.12, 0.16 and 0.20 mg/ml.

Calibration curve

2 ml of working solution was pippeted into test tubes. 10 ml of anthrone reagent was added and mixed by shaking. Tubes were covered with glass stoppers and placed immediately in boiling water bath for 12 min. The absorbance was measured at 620 nm.

Extraction of sugars and starch

Finely ground sample, weighing 0.2 g was transferred to 50 ml centrifuge tube, and added two drops of 80% hot alcohol to aid mixing. Then 5 ml of water was added and stirred. An amount of 25 ml of 80% hot alcohol was added and stirred again. It was settled aside for 5 min. and then centrifuged. The alcoholic solution transferred and the procedure was repeated by adding 30 ml of 80% hot alcohol to the residue. The two alcohol extractions were combined and evaporated under reduced pressure in a boiling water bath. The aqueous solution of sugars was then analyzed with the anthrone reagent for estimation of quantity of glucose in the test extract by comparison with the increased absorption due to 100 µg of glucose. The extraction of starch was carried out by adding 5 ml of water to the residue and 6.5 ml of 52% perchloric acid. The mixture was

stirred with glass rod for 15 min. and then centrifuged after adding 20 ml of water. Supernatant was transferred to 100 ml flask and extraction was repeated. The combined extracts were diluted to 100 ml and analyzed with anthrone reagent.

3.2.6 Determination of non-phytate (available) phosphorus

For the determination of available phosphorus, the total phosphorus content in the bran samples was analyzed following standard techniques (AOAC, 1990). The phytate phosphorus content was analyzed as per Haugh and Lantzsch (1983). The method in brief has been given below.

Preparation of reagents

I. Phytate reference solution

The sodium salt of phytic acid was obtained from sigma. Stocks solutions were prepared which contained 0.15 g sodium phytate in 100 ml distilled water. As phytase is absent, these stock solutions are stable. The reference solutions were prepared by diluting the stock solutions with HCl in a range from 3.39 to 33.9 µg ml-1 phytate phosphorus (about 1 to 10 ml stock solution and 0.73 ml HCl in each and then diluted in 100 ml). The HCl concentration in the reference solutions should be 0.2N.

II. Ferric solution

0.2 g ammonium iron sulphate.12 H_2O which was obtained from Merck, dissolved in 100 ml 2N HCl and made up to 1000 ml with distilled water.

III. 2, 2-Bipyridine solution

10 g 2, 2-bipyridine and 10 ml thioglycollic acid dissolved in distilled water and made up to 1000 ml. These solutions are stable for several months at room temperature.

Procedure

0.02 g samples were extracted with 10 ml 0.2N HCl. An aliquot of 0.5 ml of this extract was pippeted into a test tube fitted with a glass stopper. 1 ml of ferric solution was added into the tubes. The tubes were covered with the stopper and fixed with a clip. The tubes were heated in a boiling water bath for 30 min. The care was taken that tubes remained well stoppered for the first 5 min. After cooling in ice water for 15 min, the tubes were allowed to adjust to room temperature. The contents of the tube were mixed and centrifuged for 30 min. at 3000 g. 1 ml of 2, 2-bipyridine solution transferred to another test tube and added 1.5 ml of ferric solution. The contents were mixed. The absorbance was measured after a defined time (0.5-1.0 min. is recommended) at 519 nm against distilled water. Bipyridine react with the iron phytate and therefore, the colour changes with time.

3.2.7 Acid detergent fiber

The acid detergent fiber (ADF) content in the raw and processed cereals was determined as per the method of Van Soest (1963).

3.2.8 Chemical composition of dietary ingredients

Sufficient quantities of the required feed ingredients and supplements were procured from the feed storage and processing unit of the institute. The practical feed ingredients and supplements included yellow maize, wheat bran (WB), soybean meal (SBM, deoiled), rapeseed meal (RSM), deoiled rice bran (DORB), refined soybean oil and salt, dicalcium phosphate (DCP), limestone (LS), laboratory reagent grade inorganic salts (copper sulphate pentahydrate, ferrous sulphate septahydrate, manganese sulphate hydrate, potassium iodide, zinc sulphate septahydrate) to prepare trace mineral mixture, and commercial vitamin premix. The proximate composition,

phosphorus (AOAC, 1990) and calcium (Talpatra *et al.*, 1940) contents of the feed ingredients and diets were analyzed following standard techniques.

3.2.9 Enzyme analysis

The commercial multi-enzyme preparation (Brozyme) was analyzed for different enzyme activities following standard methods compiled by Sastry *et al.* (1999). The preparation was found to contain α-amylase (EC 3.2.1.1) 2000 ± 51 mIU/g; β-glucanase (EC 3.2.1.21) 150 ± 25 mIU/g; xylanase (EC 3.2.1.8) 3000 ± 48 mIU/g; carboxymethyl cellulase (EC 3.2.1.4) 40 ± 12.5 mIU/g; pectinase 150 ± 48 mIU/g; proteinase 600 ± 52 mIU/g; α-galactosidase 250 ± 38 mIU/g; β-galactosidase (EC 3.2.1.37) 200 ± 21 mIU/g; lipase 400 ± 45 mIU/g and phytase 50± 4.8 mIU/g. The activities (mIU/kg) of different enzymes in fermented bran were β-glucosidase 17566; β-D-xylosidase 49233; xylanase 1101; carboxymethyl cellulase 953; FTPase (filter paper degrading activity) 193 and α-amylase 2889.

3.3 EXPERIMENT 3: EFFECTS OF FEEDING FERMENTED WHEAT BRAN WITH OR WITHOUT ENZYME ON GROWTH AND IMMUNE RESPONSE IN BROILERS

The present experiment was conducted to assess the effect of fermentation (F) on utilization of wheat bran (WB) in diets of commercial broiler chickens (*CARIBRO*-Vishal). Day-old broiler chicks (n=256) were randomly divided into 32 groups of 8 chicks each, and 8 dietary treatments were allotted to 4 groups (replicates) in a completely randomized design. Out of the eight treatments, one was maize-soyabean based control diet (D_1). The rest of the treatments were diets consisting of two levels (5 and 7.5% part) of wheat bran either raw with/without enzymes or fermented wheat bran (Table 3.3.1).

3.3.1 Fermentation of wheat bran

The wheat bran was procured from the local market. Optimum moisture concentration for growth of *A. niger* growth was determined by visual inspection and chemical analysis. The most suitable moisture level was assessed through dry matter loss and chemical analyses (AOAC, 2000). Then, WB was soaked in autoclaved distilled water at the ratio 70:30 and mixed thoroughly. It was autoclaved at 15 psi for 15 min. Then, it was spread uniformly in a tray of 1.5 to 2.0 cm thick layer. Spore suspension was inoculated at the rate of one lac spore per kg of autoclaved WB. The polythene sheet (300 gauge) was spread over the tray after inoculation and spraying of spore suspension. The polythene sheet was folded on all the sides of the tray to create anaerobic condition for fermentation in BOD incubator. The best moisture level standardized in the earlier experiment was maintained for final fermentation. The fermented bran was then (with or without enzyme) tried on day-old broiler chicks up to their market age to find out their feeding value and improvement in feeding value, if any. They were compared simultaneously with the untreated wheat bran with or without enzyme at the same levels.

3.3.2 Experimental diets

Eight dietary treatments (Table 3.2.2 and 3.2.3) were formulated as D_1: Maize + soybean meal (control diet), D_2: wheat bran (5%) as such, D_3: wheat bran (7.5%) as such, D_4: wheat bran (0%) as such with multienzyme @ 0.5 g/kg, D_5: wheat bran (5%) as such with multienzyme @ 0.5 g/kg, D_6: wheat bran (7.5%) as such with enzyme @ 0.5 g/kg, D_7: fermented wheat bran (5%) as sole bran and D_8: fermented wheat bran (7.5%) as sole bran for 0-3 wk (starter mash) and 3-6 wk (finisher mash) growth phases. Each diet was offered to 32 broilers divided into four groups (replicates).

3.3.3 Experimental plan

Two hundred and fifty six straight run (*CARI BRO* Vishal) broiler chicks were procured from the CARI hatchery, wing banded, weighed and distributed at random to 32 groups of 8 chicks each. Each dietary treatment was offered randomly to four replicated groups of eight birds in each.

The broiler chicks were housed in battery cages fitted with linear feeder, waterers and dropping trays along with electrical heating arrangement. Standard managemental practices for brooding were followed with initial temperature of around 35°C at first week of age and gradually reduced to 21°C. Light was provided for 24 h to encourage feed intake. The birds were fed and provided with drinking water *ad lib.* Vaccination was carried out during the experiment as per schedule and other managemental practices followed remained similar in all the treatments.

Table 3.3.1: Description of the dietary treatments used in experiment 3

Diet No	Diet code	Wheat bran type	Level of WB/ FWB (%)
D_1	WB_0	Control	0
D_2	WB_5	Raw	5
D_3	$WB_{7.5}$	Raw	7.5
D_4	WB_0+E	Control + Enz	0
D_5	WB_5+E	Raw + Enz	5
D_6	$WB_{7.5}$+E	Raw + Enz	7.5
D_7	FWB_5	Fermented	5
D_8	$FWB_{7.5}$	Fermented	7.5

WB - wheat bran, FWB – Fermented wheat bran

Laboratory analysis

The representative samples of diets and feed ingredients were analyzed for proximate composition and phosphorus following standard

techniques (AOAC, 1990) and calcium by the method as described by Talpatra *et al.* (1940).

3.3.4 Response criteria

The data on various parameters with reference to growth performance, nutrient retention, immune response, carcass and meat quality, digestive organ weights and feed cost of broiler production was collected as detailed below.

3.3.4.1 Growth performance

Feed intake

The feed intake of chicks, allotted in replicates, was recorded at weekly interval from 0 to 6 weeks of age. The weighed quantity of feed was offered daily as mash and the residual feed from the individual feeding trough of each group was collected every week and weighed separately. The actual feed intake in each group of the individual dietary treatment was measured by taking due care for any loss of the feed falling outside the feeding trough due to scattering by the chicks, the average cumulative feed intake per bird was also calculated.

Body weight gain

All the chicks were weighed individually for recording their body weight on the initial day and also at weekly intervals from 0-6 weeks period. Based on body weight record, the average body weight gains under the different dietary treatments were calculated for every week, 0-3 and 3-6 weeks period and also for the entire period (0-6 weeks).

Table 3.3.2: Ingredient and nutrient composition (%) of starter diets used in experiment 3 (0-3 wk)

Ingredient	WB_0 D_1	WB_5 D_2	$WB_{7.5}$ D_3	WB_0+E D_4	WB_5+E D_5	$WB_{7.5}$+E D_6	FWB_5 D_7	$FWB_{7.5}$ D_8
Maize	51.40	49.40	46.74	51.40	49.40	46.74	51.14	48.80
DORB	3.50	0.00	0.00	3.50	0.00	0.00	0.00	0.00
Soybean meal	40.00	40.00	40.80	40.00	40.00	40.80	40.00	39.275
RSM	1.50	1.40	0.00	1.50	1.40	0.00	0.00	0.00
Wheat bran	0.00	5.00	7.50	0.00	5.00	7.50	0.00	0.00
FWB	0.00	0.00	0.00	0.00	0.00	0.00	5.00	7.50
Oil	0.00	0.70	1.50	0.00	0.70	1.50	0.25	0.90
Lime stone	0.835	1.145	1.345	0.835	1.145	1.345	1.145	1.300
DCP	1.90	1.50	1.30	1.90	1.50	1.30	1.60	1.35
DL-Methionine	0.10	0.10	0.10	0.10	0.10	0.10	0.11	0.11
Lysine	0.05	0.04	0.00	0.05	0.04	0.00	0.04	0.05
Constant*	0.715	0.715	0.715	0.715	0.715	0.715	0.715	0.715
Total	100	100	100	100	100	100	100	100
Nutrient composition								
Crude protein[1]	21.09	21.09	21.08	21.09	21.09	21.08	21.05	21.03
ME, kcal/kg[2]	2797	2795	2795	2797	2795	2795	2791	2791
Calcium[1]	1.04	1.03	1.03	1.04	1.03	1.03	1.04	1.03
Avl. P[2]	0.49	0.48	0.48	0.49	0.48	0.48	0.49	0.48
Lysine[1]	1.31	1.31	1.30	1.31	1.31	1.30	1.31	1.31
Methionine[1]	0.50	0.50	0.49	0.50	0.50	0.49	0.50	0.50
Cost, Rs/kg[2]	16.12	16.56	16.98	16.27	16.71	17.13	16.36	16.64

*Constant includes salt 0.3%, trace mineral premix 0.1%, vitamin premix 0.15%, vit. B complex & E 0.015%, choline chloride 0.05%, Toxin binder 0.05% and coccidiostat 0.05%. Trace mineral premix supplied mg / kg diet: Mg, 300; Mn, 55; I, 0.4; Fe, 56; Zn, 30; Cu, 4. The vitamin premix supplied per kg diet: Vit.A, 8250 IU; Vit. B2 5mg; Vit.D3, 1200 IU; Vit.K, 1mg. Vitamin B complex & E supplied per kg diet: Vit. B1, 4 mg; Vit. B6 8 mg; Vit.B12, 40mcg; niacin, 60mg; pantothenic acid, 40mg; Vit.E, 80 IU. Choline chloride supplied per kg diet: choline, 500mg.

WB - Wheat bran, FWB – Fermented wheat bran, RSM- Rapeseed meal, DCP-Di-calcium phosphate and E-Enzyme.

[1]Analyzed values, [2]Calculated value

Feed conversion ratio

Based on the feed intake and body weight gain data, the feed conversion ratios were calculated for each replicate, separately.

Energy and protein efficiency

The energy (metabolizable energy intake in kcal/kg: live weight gain in g) and protein efficiency (crude protein intake in g: gain in g) was calculated based on unit energy or protein consumed to unit body weight for each replicate, separately.

Mortality rate

Mortality as and when occurred was duly recorded and calculated.

Table 3.3.3: Ingredient and nutrient (%) composition of finisher diets used in experiment 2 (3-6 wk)

Ingredient	WB_0 D_1	WB_5 D_2	$WB_{7.5}$ D_3	WB_0+E D_4	WB_5+E D_5	$WB_{7.5}$+E D_6	FWB_5 D_7	$FWB_{7.5}$ D_8
Maize	58.410	52.825	50.800	58.410	52.825	50.800	53.595	51.880
DORB	0.16	0.00	0.00	0.16	0.00	0.00	0.00	0.00
Soybean meal	35.04	35.18	34.79	35.04	35.18	34.79	34.55	33.58
RSM	0.74	0.00	0.00	0.74	0.00	0.00	0.00	0.00
Wheat bran	0.00	5.00	7.50	0.00	5.00	7.50	0.00	0.00
FWB	0.00	0.00	0.00	0.00	0.00	0.00	5.00	7.50
Oil	1.95	3.50	3.50	1.95	3.50	3.50	3.30	3.57
Lime stone	0.935	1.140	1.345	0.935	1.140	1.345	1.140	1.345
DCP	1.90	1.50	1.25	1.90	1.50	1.25	1.55	1.25
DL-Methionine	0.10	0.10	0.10	0.10	0.10	0.10	0.11	0.11
Lysine	0.05	0.04	0.00	0.05	0.04	0.00	0.04	0.05
Constant*	0.715	0.715	0.715	0.715	0.715	0.715	0.715	0.715
Total	100	100	100	100	100	100	100	100
Nutrient composition								
Crude protein[1]	19.00	19.00	19.00	19.00	19.00	19.00	19.07	19.00

ME, kcal/kg[2]	3000	2999	2957	3000	2999	2957	3002	2984
Calcium[1]	1.05	1.00	1.00	1.05	1.00	1.00	1.01	1.00
Avl. P[2]	0.46	0.46	0.45	0.46	0.46	0.45	0.47	0.45
Lysine[1]	1.17	1.18	1.15	1.17	1.18	1.15	1.16	1.16
Methionine[1]	0.71	0.71	0.71	0.71	0.71	0.71	0.69	0.68
Cost, Rs/kg[2]	16.95	17.75	17.59	17.10	17.90	17.74	17.64	17.66

*Constant includes salt 0.3%, trace mineral premix 0.1%, vitamin premix 0.15%, vit. B complex & E 0.015%, choline chloride 0.05%, Toxin binder 0.05% and coccidiostat 0.05%. Trace mineral premix supplied mg / kg diet: Mg, 300; Mn, 55; I, 0.4; Fe, 56; Zn, 30; Cu, 4. The vitamin premix supplied per kg diet: Vit.A, 8250 IU; Vit. B2 5mg; Vit.D3, 1200 IU; Vit.K, 1mg. Vitamin B complex & E supplied per kg diet: Vit. B1, 4 mg; Vit. B6 8 mg; Vit.B12, 40 mcg; niacin, 60mg; pantothenic acid, 40mg; Vit.E, 80 IU. Choline chloride supplied per kg diet: choline, 500mg.

WB - Wheat bran, FWB – Fermented wheat bran, RSM- Rapeseed meal, DCP-Di-calcium phosphate and E-Enzyme.

[1]Analyzed values, [2]Calculated value

3.3.4.2 Metabolism trial

A metabolism trial of three days collection period was conducted on all birds present in each group to assess the retention of energy and nitrogen. Metabolic trial was conducted during the fourth week in the same cages, where the birds were reared from the beginning. A total collection method was employed. During this period, besides offering weighed quantity of feed, the total excreta voided during 24 h period was collected daily, weighed and a representative sample of excreta was pooled for consecutive three days and simultaneously dried in hot air oven with exhaust facility at 60°C till a constant weight was obtained. The dried excreta, feed and residue samples were ground and processed for analysis of nitrogen and gross energy contents. The representative samples of feed ingredients were analyzed for proximate composition, phosphorus (AOAC, 1990) and calcium (Talpatra *et al.,* 1940) following standard techniques. The samples of excreta and residue, were analyzed for nitrogen and gross energy.

3.3.4.3 Nutrient utilization

From the knowledge of the various nutrients present in the experimental diets and those of the excretal samples generated by birds in different dietary treatments during the balance period , the retention of the following nutrients were worked out:

- Energy metabolizability: Classical AME/GE x 100
- Nitrogen retention: (N intake-N outgo)/N intake x 100
- Dry matter metabolizability: (DM intake-DM outgo)/DM intake x 100
- Calcium retention: (Ca intake-Ca outgo)/Ca intake x 100
- Phosphorus retention: (P intake-P outgo)/P intake x 100

3.3.4.4 Caeca microbial status

On 35th day, two chicks per replicate per dietary treatment were sacrificed by cervical dislocation and caecal contents were collected in sterile vials for evaluation of total microbial load colonization. One gram caeca content was dissolved in 9 ml sterile normal saline solution (NSS).0.5 ml from 10_4 dilutions was taken in sterile Petri dish and 15-20 ml of sterile nutrient agar media was poured in each Petri dish. It was mixed gently and allowed to stand until the media solidify. Then plates were incubated in BOD incubator at 37^{o}C for 24 h and total number of colonies were counted by a colony counter (ICMSF, 1978).

$$\text{cfu/g} = \frac{\text{Total number of colony counted x Dilution factor}}{\text{Volume of aliquot taken}}$$

3.3.4.5 Carcass quality traits

At the end of the 6 weeks feeding experiment, one male and one female bird from each replicate (8 birds/treatment) were

selected as per the body weight close to mean and starved for 12 h before the actual slaughter, but the drinking water was provided ad lib. The birds were killed by improved Kosher method in which severing the jugular vein and carotid artery on one side of the neck, allowed to bleed for 1-2 min., scalded at 54°C for 2 min. in dunking scalder and defeathered mechanically for 30-60 seconds in a rotary drum picker. The carcass traits viz. eviscerated yield, edible meat yield, ready-to cook yield and giblets (liver, heart, and gizzard) were recorded and expressed in terms of per cent of live weight. Also, cut-up part yields (breast, thigh, drumsticks, back, neck and wing) were recorded and expressed in terms of per cent of eviscerated weight.

3.3.4.6 Development of immune organs

The development of immune and digestive organs was studied at 42 days of age. The bursa of fabricius and spleen were weighed and expressed as per cent of live weight.

3.3.4.7 Feed cost of broiler production

The cost of reference and test diets was calculated taking into consideration the prevailing price of individual feed ingredients, supplements, enzymes. The cost per kg body weight gain; per kg final body weight (BWT) and per kg total edible meat yield (eviscerated weight with giblet) of broilers reared under different feeding regimens of the present study was calculated based on feed consumption during the 0-42 days period.

3.3.4.8 Serum biochemical parameters

On 42nd day of experimental period, 3 ml blood was collected from eight birds in each treatment randomly and serum was

separated. The serum cholesterol (Wybenga *et al.*, 1970), triglycerides (Fossati and Lorenz, 1982) and uric acid (Pileggi *et al.*, 1972) were estimated by using respective commercially available diagnostic kits (Sigma Diagnostics Pvt. Ltd., Baroda, India).

3.3.4.9 Immunological parameters

Cell mediated immune response (CMI) to PHA-P (Phytohaemagglutinin, lectin from Phaseolus vulgaris) and humoral response to sheep red blood cell (SRBC) as influenced by different dietary treatments were assed following standard techniques as described below.

i. Cell mediated immune response

The CMI response was assessed by cutaneous basophilic hypersensitivity test *in vivo* by using PHA-P. At 22nd day post-hatch, eight birds from each treatment were selected and the skin thicknesses of both left and right foot at inter digital space between third and fourth toe was measured by micrometer. Immediately after measurements 100 mg of PHA-P suspended in 0.1 ml of phosphate buffer saline (PBS) and 0.1 ml of PBS was injected into right and left foot (acted as control), respectively. The web swellings of both the foot were measured 24 hours after injection. The response was determined by subtracting the skin thickness of first measurement from the second and the values of left foot (control) from the right foot (Corrier and De Loach, 1990).

ii. Humoral immune response to SRBC

Preparation of reagents

Alsever's solution and phosphate buffer saline (PBS) were prepared as per the composition given bellow.

Alsever's solution

Dextrose	2.05 g
Tri sodium citrate dihydrate	0.80 g
Sodium chloride	0.42 g
Citric acid	0.055g
Distilled water	100 ml

The pH of the solution was adjusted to 6.5 by addition of citric acid and stored in refrigerator at 4°C.

Phosphate buffer saline (PBS)

Sodium chloride (NaCl)	8.0 g
Potassium chloride (KCl)	0.20 g
Potassium dihydrgen phosphate (KH_2PO_4)	0.20 g
Disodium hydrogen phosphate ($Na_2HPO_4.2H_2O$)	1.44 g
Distilled water	1000 ml

The pH of the solution was adjusted to 7.2.

Procedure

Blood from jugular vein of healthy sheep was collected in Alsever's solution. The blood was centrifuged at 2500 rpm for about 10 min. The supernatant was discarded and the red blood cells were washed thrice in PBS. Suspension of SRBC (1% v/v) in PBS was prepared and stored in refrigerator at 4°C until its use. At 28th day post-hatch, 1.0 ml suspension of SRBC was injected intravenously into two birds per replicate to study the primary antibody response to SRBC. At 34th day (5 days post-immunization), 2 ml blood was collected from the jugular vein. The blood was allowed to clot, the serum was collected, and frozen (-20°C) until analyzed for the antibody titers to SRBC.

Haemagglutination test (HA test)

The antibody titer was determined by HA methods (Vander Zijpp *et al.*, 1983; Siegel and Gross, 1980). An amount of 50 µl of PBS

was distributed in each well of the micro titer plate. Fifty micro liters of serum was added in the first well. Two fold serial dilutions were made up to row 11 and row 12 was kept as control. Fifty micro liters of 1% SRBC was added in each well and mixed by gentle tapping. The plates were covered and then kept at 37°C for 1 h for incubation. The plates were read under bright light. The reciprocal of highest dilution showing clear agglutination was the end titer. The titers were expressed as log 2.

3.3.4.10 Sensory evaluation

The sensory test was conducted for the evaluation of the broiler meat as influenced by the feeding of wheat bran as such, wheat bran with or without enzyme and fermented wheat bran to the broiler chickens. For this purpose, one bird from each dietary treatment was randomly selected and sacrificed as per the conventional standard slaughter technique. The ready-to-cook meat pieces of breast and right thigh region of sacrificed bird under different dietary treatment were cooked simultaneously under pressure (1 kg/cm^3 for 10 min.), cooled to ambient temperature prior to subjecting the cooked meat for sensory evaluation. The cooked meat was served for evaluation of its sensory quality by a panel of semi-trained judges using a 10-point hedonic scale on the standard proforma for sensory attributes of the meat such as appearance, tenderness, texture, juiciness, flavour and overall acceptability.

3.3 EXPERIMENT 4: EFFECT OF FERMENTATION WITH OR WITHOUT ENZYMES ON UTILIZATION OF DE-OILED RICE BRAN IN BROILERS

In this experiment, the effect of fermentation on utilization of de-oiled rice bran (DORB) in diets of CARI coloured broiler strain

was assessed. Day-old broiler chicks (n=256) were randomly divided into 32 groups of 8 chicks each, and 8 dietary treatments were allotted to 4 groups (replicates) in a completely randomized design. Out of the eight treatments, one was maize-soybean based control diet (D_1). The rest of the treatments were diets consisting of two levels (5 and 7.5% part) of de-oiled rice bran either raw with/without enzymes or fermented de-oiled rice bran (Table 3.4.1).

3.4.1 Fermentation of de-oiled rice bran

The de-oiled rice bran (DORB) was procured from the local market and fermented by using *A. niger* through solid substrate fermentation. Optimum moisture concentration for growth of *A. niger* growth was determined by visual inspection and chemical analysis as described in experiment 2. The most suitable moisture level was assessed through dry matter loss and chemical analyses (AOAC, 2000). Then, DORB was soaked in autoclaved distilled water in the ratio 50:50 and mixed thoroughly. It was autoclaved at 15 psi for 15 min. Then, it was spread uniformly in a tray of 1.5 to 2.0 cm thick layer. Spore suspension was inoculated at the rate of one lac spore per kg of autoclaved WB. The polythene sheet (300 gauges) was spread over the tray after inoculation and spraying of spore suspension. The polythene sheet was folded on all the sides of the tray to create anaerobic condition for fermentation in BOD incubator. The fermented bran was then (with or without enzyme) tried on day-old broiler chicks up to their market age to find out their feeding value and improvement in feeding value, if any. They were compared simultaneously with the untreated de-oiled rice bran with or without enzyme at the same levels.

3.4.2 Experimental diets

Eight dietary treatments (Table 3.2.2 and 3.2.3) were formulated as D_1: Maize + soybean meal (control diet), D_2: de-oiled

rice bran (5%) as such, D3: de-oiled rice bran (7.5%) as such, D_4: de-oiled rice bran (0%) as such with multienzyme @ 0.5 g/kg, D_5: de-oiled rice bran (5%) as such with multienzyme @ 0.5 g/kg, D_6: de-oiled rice bran (7.5%) as such with multienzyme @ 0.5 g/kg, D_7: fermented de-oiled rice bran (5%) as sole bran and D8: fermented de-oiled rice bran (7.5%) as sole bran for 0-3 wk (starter mash) and 3-6 wk (finisher mash) growth phases. Each diet was offered to 32 broilers divided into four groups (replicates).

Table 3.4.1: Description of the dietary treatments used in experiment 4

Diet No.	Diet code	De-oiled rice bran type	Level of DORB/ FDORB (%)
D_1	$DORB_0$	Control	0
D_2	$DORB_5$	Raw	5
D_3	$DORB_{7.5}$	Raw	7.5
D_4	$DORB_0$+E	Control + Enz	0
D_5	$DORB_5$+E	Raw + Enz	5
D_6	$DORB_{7.5}$+E	Raw + Enz	7.5
D_7	$FDORB_5$	Fermented	5
D_8	$FDORB_{7.5}$	Fermented	7.5

DORB - De-oiled rice bran, FDORB –Fermented de-oiled rice bran.

3.4.3 Experimental plan

Sufficient number of fertile eggs of CARI *coloured* broiler strain were procured from broiler breeding farm of CARI, Izatnagar and incubated. After hatching, day-old chicks (n=256) were randomly selected, weighed and distributed into 32 groups of 8 chicks each. They were offered eight dietary treatments. Each dietary treatment group had four subgroups (replicates) i.e. 32 chicks. A basal ration adequate in all nutrients was formulated based on maize as a major source of energy and seven dietary (iso-caloric) treatments (Table 3.4.1) were formulated with two levels (5 and 7.5%) of de-oiled rice

bran and its fermented forms i.e. as such, as such supplemented with or without enzymes, fermented de-oiled rice bran (as described earlier). The dietary treatments are presented in Tables 3.4.2 and 3.4.3. All the chicks were reared in groups in electrically heated battery brooders with wire-mesh floor from 0 to 42 d of age.

Table 3.4.2: Ingredient and nutrient composition (%) of starter diets used in experiment 4 (0-3 wk)

Ingredient	DB_0 D_1	DB_5 D_2	$DB_{7.5}$ D_3	DB_0+E D_4	DB_5+E D_5	$DB_{7.5}$+E D_6	FDB_5 D_7	$FDB_{7.5}$ D_8
Maize	52.400	47.410	44.495	52.400	47.410	44.495	49.285	48.800
Soybean meal	39.500	40.000	40.100	39.500	40.000	40.100	40.800	39.275
RSM	4.575	3.185	2.700	4.575	3.185	2.700	1.000	0.500
DORB	0.00	5.00	7.50	0.00	5.00	7.50	0.00	0.00
FDORB	0.00	0.00	0.00	0.00	0.00	0.00	5.00	7.50
Oil	0.00	1.00	1.70	0.00	1.00	1.70	0.40	0.80
Lime stone	1.00	1.00	1.20	1.00	1.00	1.20	1.20	1.30
DCP	1.70	1.60	1.50	1.70	1.60	1.50	1.50	1.30
DL-Methionine	0.08	0.09	0.09	0.08	0.09	0.09	0.10	0.10
Lysine	0.03	0.00	0.00	0.03	0.00	0.00	0.00	0.00
Constant*	0.715	0.715	0.715	0.715	0.715	0.715	0.715	0.715
Total	100	100	100	100	100	100	100	100
Nutrient composition								
Crude protein[1]	21.48	21.49	21.46	21.48	21.49	21.46	21.48	21.03
ME, kcal/kg[2]	2829	2817	2815	2829	2817	2815	2810	2811
Calcium[1]	1.04	1.03	1.03	1.04	1.03	1.03	1.06	1.04
Avl. P[2]	0.45	0.46	0.45	0.45	0.46	0.45	0.45	0.44
Lysine[1]	1.27	1.27	1.28	1.27	1.27	1.28	1.30	1.30
Methionine[1]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Cost, Rs/kg[2]	16.14	16.51	16.78	16.29	16.66	16.93	16.25	16.31

*Constant includes salt 0.3%, trace mineral premix 0.1%, vitamin premix 0.15%, vit. B complex & E 0.015%, choline chloride 0.05%, Toxin binder 0.05% and coccidiostat 0.05%. Trace mineral premix supplied mg / kg diet: Mg, 300; Mn, 55; I, 0.4; Fe, 56; Zn, 30; Cu, 4. The vitamin premix supplied per kg diet: Vit.A, 8250 IU; Vit. B2, 5mg; Vit.D3, 1200 IU; Vit.K, 1mg. Vitamin B complex & E supplied per kg diet: Vit. B1, 4 mg; Vit. B6, 8 mg; Vit.B12, 40 mcg; niacin, 60mg; pantothenic acid, 40mg; Vit.E, 80 IU. Choline chloride supplied per kg diet: choline, 500mg.

DORB - De-oiled rice bran, FDORB – Fermented de-oiled rice bran, RSM- Rapeseed meal, DCP-Di-calcium phosphate and E-Enzyme.

[1]Analyzed values, [2]Calculated value

Table 3.4.3: Ingredient and nutrient (%) composition of finisher diets used in experiment 4 (3-6 wk)

Ingredient	DB_0 D_1	DB_5 D_2	$DB_{7.5}$ D_3	DB_0+E D_4	DB_5+E D_5	$DB_{7.5}$+E D_6	FDB_5 D_7	$FDB_{7.5}$ D_8
Maize	61.000	55.445	52.52	61.000	55.445	52.520	56.605	54.185
Soybean meal	35.305	34.800	34.475	35.305	34.800	34.475	34.000	33.400
DORB	0.00	5.00	7.50	0.00	5.00	7.50	0.00	0.00
FDORB	0.00	0.00	0.00	0.00	0.00	0.00	5.00	7.50
Oil	0.00	1.25	1.90	0.00	1.25	1.90	0.80	1.30
Lime stone	1.10	1.00	1.20	1.10	1.00	1.20	1.20	1.30
DCP	1.80	1.70	1.60	1.80	1.70	1.60	1.60	1.50
DL-Methionine	0.08	0.09	0.09	0.08	0.09	0.09	0.08	0.10
Lysine	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Constant*	0.715	0.715	0.715	0.715	0.715	0.715	0.715	0.715
Total	100	100	100	100	100	100	100	100
Nutrient composition								
Crude protein[1]	19.07	19.06	19.01	19.07	19.06	19.01	19.04	19.02
ME, kcal/kg[2]	2905	2904	2899	2905	2904	2899	2900	2899
Calcium[1]	1.07	1.02	1.07	1.07	1.02	1.07	1.06	1.07
Avl. P[2]	0.45	0.46	0.45	0.45	0.46	0.45	0.45	0.46
Lysine[1]	1.14	1.14	1.14	1.14	1.14	1.14	1.12	1.12
Methionine[1]	0.45	0.46	0.45	0.45	0.46	0.45	0.45	0.46
Cost, Rs/kg[2]	15.66	16.14	16.35	15.81	16.29	16.50	15.77	15.96

*Constant includes salt 0.3%, trace mineral premix 0.1%, vitamin premix 0.15%, vit. B complex & E 0.015%, choline chloride 0.05%, Toxin binder 0.05% and coccidiostat 0.05%. Trace mineral premix supplied mg / kg diet: Mg, 300; Mn, 55; I, 0.4; Fe, 56; Zn, 30; Cu, 4. The vitamin premix supplied per kg diet: Vit.A, 8250 IU; Vit. B2 5mg; Vit.D3, 1200 IU; Vit.K, 1mg. Vitamin B complex & E supplied per kg diet: Vit. B1, 4 mg; Vit. B6 8 mg; Vit.B12, 40 mcg; niacin, 60mg; pantothenic acid, 40mg; Vit.E, 80 IU. Choline chloride supplied per kg diet: choline, 500mg.

DORB - De-oiled rice bran, FDORB – Fermented de-oiled rice bran, RSM- Rapeseed meal, DCP-Di-calcium phosphate and E-Enzyme.

[1]Analyzed values, [2]Calculated value

3.4.4 Response criteria

As described in experiment 3.

3.5 STATISTICAL ANALYSIS

Individual observation was unit of measurement for weight gains, immune response, gastrointestinal tract development, and serum biochemical profile data. The pen average was the unit of measurement for FCR, Feed intake, energy and protein utilization, cost of production. The data obtained from the above experiments were subjected to statistical analysis as per standard procedures of Snedecor and Cochran (1989) and Duncan's multiple range test (Duncan, 1955) as well as Tukey test for verifying significance of treatment means.

❖❖❖

Results and Discussion...

Results and Discussion

The results of the four experiments, conducted to optimize the moisture level for better growth of *A. niger* on WB and DORB and evaluate the nutritional and feeding value of fermented WB and DORB with or without enzymes in terms of nutrient availability, growth performance, nutrient utilization, caeca microbial count, carcass characteristics, organoleptic quality of meat, serum bio-chemicals, immune-responsiveness and feed cost of broiler production, are presented and discussed in this chapter.

4.1 EXPERIMENT 1: OPTIMIZING MOISTURE LEVEL FOR GROWTH OF *A. NIGER* ON WHEAT BRAN AND DE-OILED RICE BRAN SUBSTRATES

4.1.1 Standardization of fermentation conditions of WB

The results of the *in vitro* trials conducted to find out optimum moisture level for better growth of *A. niger* fungus are presented in the Table 4.1.1, 4.1.2 and 4.1.3 and the analyses of variance of the same in Tables 4.1.1a, 4.1.2a and 4.1.3a (given as Annexure), respectively. Visual growth of *A. niger* on WB by maintaining different levels of moisture and spores inoculation at the rate of 1 lac spores/ kg of WB at constant temperature (37°C) and duration (72 h) are depicted in the plates 4.1.1 to 4.1.6.

The DM loss increased gradually with the increase in moisture level in WB substrate. Amongst the all fermented groups,

lowest DM loss (16.98%) was noted in water soaked WB at the ratio of 70:30 (w/v) and highest DM loss of 20.48% was found in water soaked WB at the ratio of 50:50 (w/v). The CP content of all FWB increased as compared to their raw counterpart. The CP content of FWB increased with the decrease in moisture level in substrate but the trend was inconsistent. Amongst the treatment groups highest CP (19.78%) was found in FWB from 70:30 ratio (w/v) and lowest CP (18.31%) in the ratio of 60:40 (w/v). Enhancement of CP content of FWB with 70:30 (w/v) ratio was 32.84% as compared to raw counterpart. Moreover, increase in moisture level of substrate group resulted decrease in CF of FWB except that with 60:40 (w/v). Lowest CF (8.88%) was found in FWB prepared with DM to water ratio of 50:50 (w/v) as compared to FWB with other ratios. Highest CF (11.35%) was found in FWB of 60:40 ratio (w/v). The decrease in fiber content of FWB with ratio of 70:30 (w/v) was about 22.55% as compared to raw WB. The EE content increased linearly in FWB with decreased moisture level. Highest EE (2.96%) was observed in FWB from 70:30 (w/v) and lowest EE (1.34%) from 50:50 (w/v). NFE content of all the FWB decreased as compared to raw WB. As moisture level in substrate type of WB decreased, NFE content decreased gradually. Highest NFE (62.10%) were noticed in WB substrate soaked with water at the ratio of 50:50 (w/v) and lowest (61.01%) 70:30 (w/v). Fermentation resulted in decreased NFE content than raw. There was increase in GE of WB substrate after fermentation as compared raw WB. A linear decline in GE of substrate groups was evident when the moisture level in substrate group decreased during fermentation. Highest GE (4659 kcal/ kg) was found in FWB, soaked at the ratio of 70:30 (w/v) and lowest GE (4623 kcal/kg) in 50:50 (w/v). Due to fermentation, there was enhancement of GE content of WB and FWB from 70:30 (w/v) had 3.33% more GE as compared to raw WB.

Table 4.1.1: Chemical composition (%DM) of wheat bran (WB) as influenced by different fermentation conditions

Substrate types	Bran: Water	DM loss*	CP	CF	EE	NFE	GE** (kcal/kg)
WB	Raw	-	14.89^{b}	13.04^{a}	2.36^{b}	62.48	4504^{b}
FWB	(70:30)	16.98^{c}	19.78^{a}	10.10^{b}	2.96^{a}	61.01	4659^{a}
FWB	(60:40)	18.74^{b}	18.31^{a}	11.35^{b}	1.61^{c}	62.07	4636^{a}
FWB	(50:50)	20.48^{a}	19.63^{a}	8.88^{c}	1.34^{c}	62.10	4623^{a}
SEM		0.453	0.630	0.500	0.199	0.417	19.926
Stast. significance		P<0.01	P<0.01	P<0.01	P<0.01	NS	P<0.01

Values bearing different superscripts within a column differ significantly.
NS- Non-significant (P>0.05)
*Note: Observations are average of five replicates and **Analysed value
WB-Wheat bran and FWB-Fermented wheat bran.

Fermentation resulted in increased TA, AIA and P content with increase in moisture level of substrate as compared to control. Highest TA (8.06%), AIA (1.52%) and P (1.49%) were found in FWB, soaked at the ratio of 50:50 (w/v) and lowest TA (6.14%), AIA (1.16%) and P (1.45%) were noticed in FWB at the ratio of 70:30 (w/v). Growth of *A. niger* was apparently higher in WB substrate when soaked with water at the ratio of 70:30 (w/v) than at the ratio of 60:40 (w/v) or 50:50 (w/v) at constant temperature (37°C) and duration (72 h). Aflatoxin screening and urease activity estimation in substrates revealed less than 0.020 ppm of aflatoxins and 0.09 mg N/g/minute at 30°C urease activity, which are considered safe for poultry. Therefore, based on all above results, the WB soaked with water at the ratio of 70:30 (w/v) was used for solid state fermentation for use in feeding trial as an ingredient in the experimental broiler rations.

Table 4.1.2: Mineral composition (%DM) of wheat bran (WB) as influenced by different fermentation conditions

Substrate types	Bran:Water	TA	AIA	Ca	P
WB	Raw	7.23	0.70^{c}	0.36^{a}	1.22^{b}
FWB	(70:30)	6.14	1.16^{b}	0.28^{b}	1.45^{a}
FWB	(60:40)	6.66	1.26^{b}	0.26^{b}	1.47^{a}
FWB	(50:50)	8.06	1.52^{a}	0.27^{b}	1.49^{a}
SEM		0.373	0.093	0.015	0.036
Stast. significance		NS	P<0.01	NS	P<0.01

Values bearing different superscripts within a column differ significantly.
NS- Non-significant (P>0.05)
WB-Wheat bran and FWB-Fermented wheat bran.

The present observations on optimization of fermentation conditions could not be ascertained as no information is available in literature, except with rumen filtrate. However, fermentation of WB with rumen filtrate at room temperature for a specified time period resulted in increase of CP and EE content from 13.6 to 14.2% and from 3.95 to 7.24%, respectively, while CF content decreased from 12% to 9.5%, GE from 3905.4 to 3599 kcal/g and TA content from 4.0 to 3.9% (Darwazeh, 2010). However, Dinani (2009) observed moderate growth of *A. niger* on toasted guar meal soaked with water at the ratio of 70:30 (w/v) with inoculation of 1 lac spores/kg of substrate at 37°C for 72 h. Nevertheless, Zamora and Veum (1979) and Mathivanan *et al.* (2006) studied fermentation on SBM and observed the better growth of *Aspergillus sp.* in SBM when soaked with water at the ratio 25:75 (w/v) at 37°C for 24 and 48 h, respectively. In contrary, Verma and McNab (1982) reported better growth of *A. niger* in guar meal to water ratio of 30:70 and at 30°C for 60 h. Enhancement of CP, TP and GE content were found from 41.48 to 54.31%; 0.42 to 0.51% and from 4858 to 4862 kcal/kg, respectively in FTGM (70:30) and NFE content of FTGM decreased from 39.67 to 24.43% as compared to Raw TGM (Dinani,

2009). Saima and Hasmi (1999) studied the suitability of WB as a substrate for the production of biomass protein and to assess its nutritive value through chemical analysis. They found that biomass protein of WB increased from 12.23 to 35.67 % and NFE decreased from 60.00 to 14.65%. In their experiment CP content was increased by 23.44% whereas in the present study CP content increased about 4.89%. *A. niger* acts as single cell protein (SCP) and releases proteases during fermentation which are possible causes of increased CP content in FWB. The aflatoxin in all types of WB above safe levels of 20 ppb was recommended by FDA (Bhatti *et al.*, 2001). The urease activity as an indirect way of measuring trypsin inhibitor was within an acceptable range of 0.05-0.2 unit (Leeson and Summers, 2001; Chee *et al.*, 2005). It is therefore, obvious that SSF enhanced the nutritive value of WB.

Table 4.1.3: Growth of *Aspergillus Niger (A. Niger)* spores, aflatoxin and urease activity of wheat bran (WB) as influenced by different fermentation conditions

Substrate types	Bran:Water	Growth of *A. Niger*	Aflatoxin (ppm)	Urease activity (mgN/g/ minute at 30^{0}C)
WB	Raw	-	0.020	-
FWB	(70:30)	+++	0.011	0.09
FWB	(60:40)	++	0.015	0.08
FWB	(50:50)	++	0.017	0.08
SEM		-	0.001	0.005
Stast. significance		-	NS	NS

NS- Non-significant (P>0.05); +low; ++moderate; +++high

4.1.2 Standardization of fermentation conditions of DORB

The results of the *in vitro* trials conducted to find out optimum moisture level for better growth of *A. niger* fungus in DORB as substrate are presented in the table 4.1.4, 4.1.5 and 4.1.6 and the

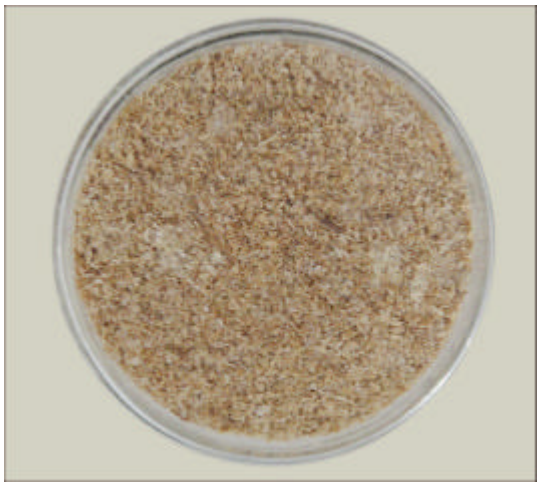


Plate 4.1.1 : WB (Raw)

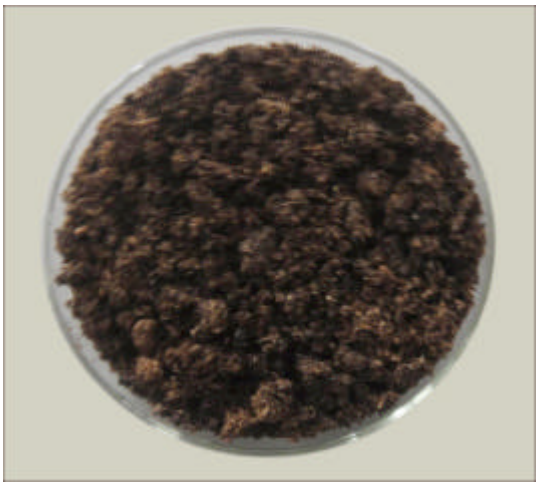


Plate 4.1.2 : FWB (70:30)

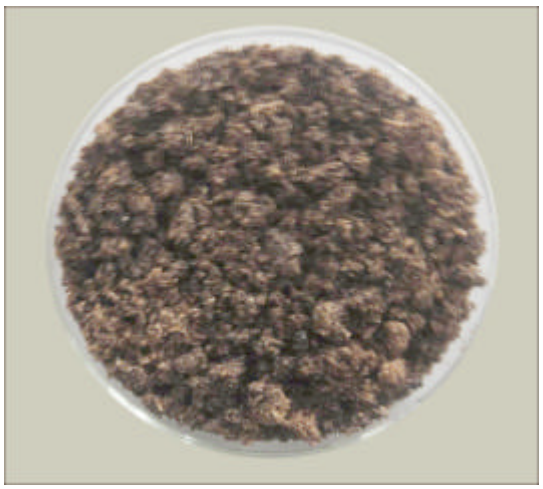


Plate 4.1.3 : FWB (60:40)

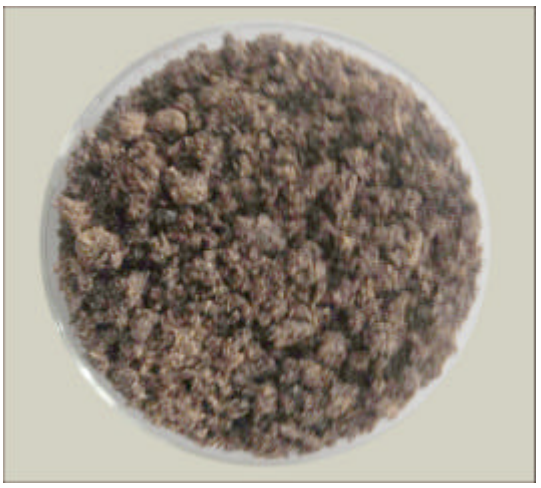


Plate 4.1.4 : FWB (50:50)

Plate 4.1.5 : WB (Raw) without fermentation

Plate 4.1.6 : WB (70:30) after fermentation and drying

analyses of variance of the same in Tables 4.1.4a, 4.1.5a and 4.1.6a (given as Annexure), respectively. Visual growth of *A. niger* on DORB by maintaining different levels of moisture with inoculation of spores at the rate of 1 lac spores/kg of DORB at constant temperature (37°C) and duration (72 h) is depicted in the plates 4.1.7 to 4.1.12. As moisture level in the substrate (DORB) increased, the DM loss decreased gradually. There was no significant difference in DM loss due to fermentation. Amongst all fermented groups, lowest DM loss (21.13%) was noted in FDORB soaked with water at the ratio of 50:50 (w/v), whereas; the highest DM loss (25.41%) was observed in FDORB soaked with water at the ratio of 70:30 (w/v). As moisture level of substrate increased; the CP content also increased compared to raw counterpart. Highest CP (18.56%) was found in FDORB soaked with water at the ratio of 50:50 (w/v), while the lowest CP (17.66%) was noticed in FDORB soaked with water at the ratio of 60:40 (w/v); though, the CP content of treatment groups remained higher than their raw counterpart. Enhancement of CP content in FDORB soaked with water at the ratio 50:50 (w/v) may be due to Aspergillus growth, which is about 27.47% higher as compared to raw DORB. Conversely, the CF content of FDORB was found to be decreased. The CF content of FDORB ranged from 7.38% (50:50 w/v) to 12.02% (60:40 w/v). The decrease in fiber content in FDORB soaked with water at the ratio of 50:50 (w/v) was about 42.57% as compared to its raw counterpart. There was gradual increase in EE content of FDORB with the increase in moisture level. Highest EE (1.78%) was found in FDORB soaked with water at the ratio of 50:50 (w/v) and lowest EE (1.36%) in FDORB soaked with water at the ratio of 70:30 (w/v). There was steady rise in NFE content of FDORB with increase in moisture level. However, the values were lower than untreated DORB except when soaked with water at the ratio of 50:50 (w/v).

Table 4.1.4: Chemical composition (%DM) of de-oiled rice bran (DORB) as influenced by different fermentation conditions

Substrate types	Bran: Water	DM loss*	CP	CF	EE	NFE	GE** (kcal/kg)
DORB	Raw	-	14.56^{b}	12.85^{a}	1.13^{c}	60.58^{a}	3881^{c}
FDORB	(70:30)	25.41	18.36^{a}	8.38^{b}	1.36^{b}	55.88^{b}	3945^{ab}
FDORB	(60:40)	24.37	17.66^{a}	12.02^{a}	1.58^{a}	57.60^{b}	3927^{bc}
FDORB	(50:50)	21.13	18.56^{a}	7.38^{b}	1.78^{a}	61.16^{a}	3986^{a}
SEM		1.006	0.550	0.719	0.079	0.717	13.209
Stast. significance		NS	P<0.01	P<0.01	P<0.01	P<0.01	P<0.01

Values bearing different superscripts within a column differ significantly.
NS- Non-significant (P>0.05)
*Note: Observations are average of five replicates and **Analysed value
DORB-De-oiled rice bran and FDORB-Fermented de-oiled rice bran

There was increase in GE of DORB after fermentation as compared raw DORB. As moisture level of FDORB increased, their GE also increased except in FDORB soaked with water at 60:40 (w/v). Highest GE (3986 kcal/ kg) was found in FDORB soaked with water at the ratio of 50:50 (w/v) and lowest GE (3927 kcal/kg) in FDORB soaked with water at the ratio of 60:40 (w/v). The enhancement of GE content of FDORB soaked with water at the ratio of 50:50 (w/v) was about 2.71% as compared to raw DORB.

TA and AIA increased as moisture level of FDORB decreased during fermentation when compared with raw DORB. Highest TA (16.02%) and AIA (3.03%) were found in FDORB soaked with water at the ratio of 70:30 (w/v). Lowest TA (11.12%) and AIA (2.10%) were noticed in FDORB soaked with water at the ratio of 50:50 (w/v).

Table 4.1.5: Mineral composition (%DM) of de-oiled rice bran (WB) as influenced by different fermentation conditions

Substrate types	Bran: Water	TA	AIA	Ca	P
DORB	Raw	10.88^{q}	2.06^{q}	0.30^{q}	1.52^{p}
FDORB	(70:30)	16.02^{p}	3.03^{p}	0.34^{q}	1.28^{q}
FDORB	(60:40)	11.13^{q}	2.10^{q}	0.32^{q}	1.58^{p}
FDORB	(50:50)	11.12^{q}	2.10^{q}	0.47^{p}	1.36^{q}
SEM		0.705	0.134	0.022	0.039
Stast. significance		P<0.01	P<0.01	P<0.01	P<0.01

Values bearing different superscripts within a column differ significantly.
NS- Non-significant (P>0.05)
DORB-De-oiled rice bran and FDORB-Fermented de-oiled rice bran

Further, as moisture level of FDORB increased, the Ca level also increased. The Ca content in FDORB (70:30 and 60:40) was comparable with raw DORB with exception of FDORB soaked with water at the ratio of 50:50 (w/v); which was significantly higher (0.47%). Whereas, P content of substrate group increased as moisture level of substrate increased. Highest P content (1.58%) was found in FDORB soaked with water at the ratio of 60:40 (w/v) and lowest (1.28%) in FDORB soaked with water at the ratio of 70:30 (w/v). *A. niger* growth was better in FDORB soaked with water at the ratio of 50:50 (w/v) than other ratios tested at constant temperature (37°C) duration (72 h) and spores inoculation @ 1 lac spores/kg of DORB). Aflatoxin screening and estimation of urease activity of substrate groups showed that all the groups contained aflatoxin less than 0.027 ppm and urease activity less than 0.05 mg N/g/minute at 30°C, which are considered safe for poultry ration. The aflatoxin in all types of DORB above safe levels of 20 ppb was recommended by FDA (Bhatti *et al.,* 2001). The urease activity as an indirect way of measuring trypsin inhibitor was within an acceptable range of 0.05-0.2 unit

(Leeson and Summers, 2001; Chee *et al.*, 2005). In view of aforesaid facts, FDORB soaked with water at the ratio of 50:50 (w/v) was used for solid substrate fermentation for subsequent feeding experiment.

Present results are in agreement with Silveira and Badiale-Furlong (2009), who also reported better growth of *A. niger* at 50:50 ratio of DORB and water, and spores inoculation of 4 x 10^6/g at 30°C for 72 h. The present study also corroborated with the observations of Anupama and Ravindra (2001) on fermentation conditions of DORB as substrate. Nevertheless, Dinani (2009) who observed that higher growth of *A. niger* on toasted guar meal and economic fermentation was found to be toasted guar meal soaked with water at the ratio of 50:50 (w/v) and spores inoculation at the rate of 1 lac spores/kg of substrate at 37°C for 72 h.

Table 4.1.6: Growth of *Aspergillus Niger (A. Niger)* spores, aflatoxin and urease activity of de-oiled rice bran (DORB) as influenced by different fermentation conditions

Substrate types	Bran:Water	Growth of *A. Niger*	Aflatoxin (ppm)	Urease activity (mgN/g/ minute at 30^{0}C)
DORB	Raw	-	0.027	-
FDORB	(70:30)	++	0.024	0.07
FDORB	(60:40)	++	0.022	0.05
FDORB	(50:50)	+++	0.021	0.06
SEM		-	0.001	0.009
Stast. significance		-	NS	NS

Note: Observations are average of three replicates (Mean)
NS- Non-significant (P>0.05); +low; ++moderate; +++high

Anupama and Ravindra (2001) studied the suitability of DORB as a best substrate for a higher yield of single cell protein production from *A. niger* and found that biomass protein of DORB increased from 2 to 12.48% when it was soaked with water at the ratio 50:50 (w/v)

Plate 4.1.7 : DORB (Raw)

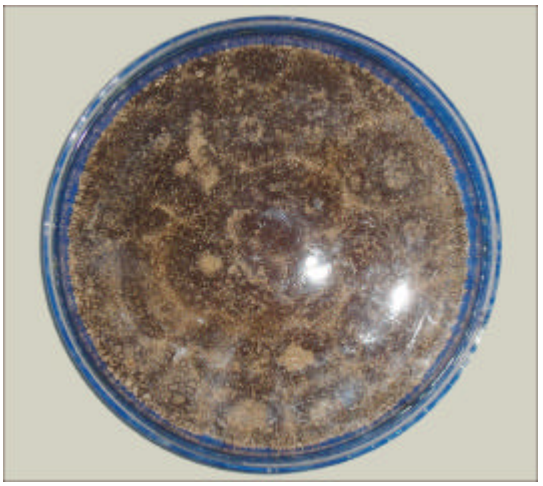


Plate 4.1.8 : FDORB (70:30)

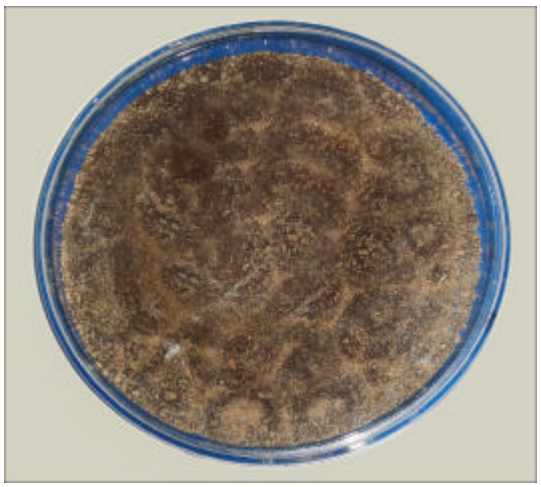


Plate 4.1.9 : FDORB (60:40)

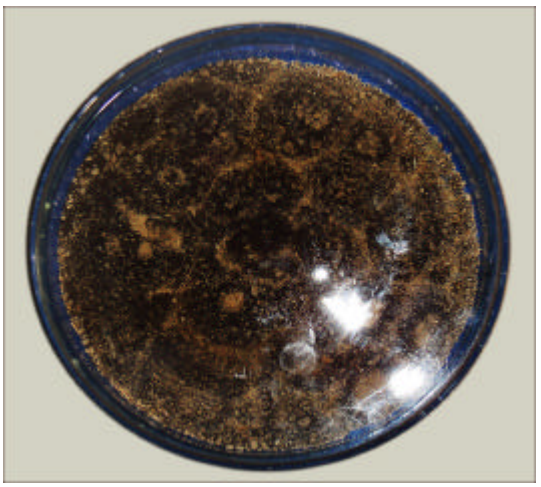


Plate 4.1.10 : FDORB (50:50)

Plate 4.1.11 : DORB (Raw) without fermentation

Plate 4.1.12 : DORB (50:50) after fermentation and drying

and incubated with spore suspension at 28°C for 8 days. Rudravaram *et al.* (2006) studied the optimization and effect of different inoculums size (10^3 to 10^{10} spores / g DORB) on protein enrichment of alkali treated DORB soaked with water at the ratio 60:40 (w/v); particle size 0.3 mm; pH 6.0 and spores concentration 10^9 spores/g substrate at 28°C for 72 h. by SSF using *A. oryzae* MTCC 1846. The protein enrichment of alkali treated DORB was from 9.20 to 18.12% after fermentation. But among the various nitrogen sources tested, ammonium sulfate (0.6% w/w) showed maximum protein enrichment 24.30% when the mineral solution and different nitrogen sources were added at 2% level. Cellulose was found to be reduced from 39 to 18%. The compositions of essential amino acids (tryptophan, lysine, threonine and cystiene) in DORB were 0.14, 0.46, 0.12 and 0.24%, respectively. The amino acids tryptophan, lysine, threonine and cystiene after treatment were found to be increase by 63, 80, 86 and 41%, respectively. Amount of reducing and total sugars increased by 80 and 83.40%, respectively, with fermentation on DORB. The increase in protein was due to high fungal proteins formed in the course of fermentation (Daubresse *et al.*, 1987). Durand and Chereau (1988) reported 69.20% of protein enrichment with sugar beet pulp. The increase in reducing and total sugars was much higher than found by Daubresse *et al.* (1987) working with cassava using *Rhizopus oryzae* MUCL 28627. Conversely, Verma and McNab (1982) reported that better growth of *A. niger* in guar meal and water at the ratio of 30:70 (w/v) at 30°C for 60 h. The CP content in defatted RB increased either from 19.2 to 20.6% or from 19.2 to 32.4% by using *Aspergillus oryzae* and *Rhizopus* species, respectively (Silveira and Badiale-Furlong, 2009). Dinani (2009) did notice similar effect when fermentation of toasted guar meal with water (70:30) and did raise DM loss and lower CF

content from 42.46 to 28.94%. Enhancement of CP, TP and GE content were found from 41.48 to 59.98%; 0.42 to 0.50% and from 4858 to 4855 kcal/kg, respectively in FTGM (50:50) and NFE content of FTGM decreased from 39.67 to 18.70% as compared to Raw TGM.

In present study CP content increased about 4%. Presence of water in the substrate makes the nutrients more easily accessible for fungal growth (Benazir *et al.*, 2011). Moreover, water has an impact on physico-chemical properties of the substrate, which in turn affect enzyme production (Pandey *et al., 1*999). Too much water adversely affects oxygen diffusion in the substrate (Chisti, 1999). The moisture content of the substrate FDORB in the present study was between 30-50%. Previous studies on the effect of moisture content on fungal growth and utilization of the substrate by Ghildyal *et al.* (1981) stated the optimum moisture content as between 40-70%. This was also in accordance with Chutmanop *et al.* (2005). Also it may be due to difference in fermentation condition and better growth of *A. niger* since high concentration of spores (1 lac spore per kg of DORB) was inoculated and also incubation temperature was higher (at 37°C) as compared to Anupama and Ravindra (2001). Further, urease activity reduction may be due to autoclaving and drying of FDORB at 57°C for 1-2 days. *A. niger* acts as single cell protein (SCP) and releases maximum yield of proteases at 72 h of incubation in a solid substrate fermentation process with RB as the substrate (Benazir *et al.*, 2011) which are possible causes of increased crude protein in FDORB. FDA revised action levels for aflatoxins to 20 ppb for all foods including animal foods. The aflatoxin in all types of DORB above safe levels of 20 ppb was recommended by FDA (Bhatti *et al.*, 2001). The urease activity as an indirect way of measuring trypsin inhibitor was within an acceptable range of 0.05-0.2 unit (Leeson and Summers, 2001). It is therefore, obvious that solid substrate fermentation enhanced the nutritive value of DORB.

4.2 EXPERIMENT 2: EFFECT OF SOLID SUBSTRATE FERMENTATION OF WHEAT BRAN AND DE-OILED RICE BRAN ON NUTRIENT AVAILABILITY

In this experiment, the FWB and FDORB as well as WB and DORB as such were evaluated for nutrient availability in terms of dry matter, energy metabolizability, *in-vitro* pepsin-pancreatin digestibility, available carbohydrates, acid detergent fiber and available phosphorus. The results are given as below:

4.2.1 Chemical composition of feed ingredients used in feeding trials

The chemical composition of feed ingredients (%, DM) used in experimental trials have been presented in Tables 4.2.1. The WB contained more protein, fat and fiber than DORB. DORB was found to contain 1.52% P content whereas, WB had 20% lower P content. The GE content of WB and DORB employed in this study were 4504 and 3881 kcal/kg, respectively. In untreated raw ingredients, the pH was near to neutral and due to fermentation it decreased and became acidic (Abalaka and Daniyan, 2010).

Table 4.2.1: Chemical composition of ingredients (%, DM) used in feeding trials

Ingredients	CP	CF	EE	TA	Ca	P	GE
Maize	9.56	3.30	3.27	2.11	0.24	0.27	4208
SBM	46.37	7.49	1.53	8.65	0.58	0.99	4225
RSM	36.65	8.81	4.09	7.15	0.85	1.72	4232
WB (Raw)	14.89	13.04	2.36	7.23	0.36	1.22	4504
DORB (Raw)	14.56	12.85	1.13	10.88	0.30	1.52	3881
LSP	-	-	-	-	34.32	-	-
DCP	-	-	-	-	29.25	16.76	-

The ingredients utilized in all the trials are maize (yellow), DORB (deoiled rice bran),

SBM - (soybean meal, solvent ext.), RSM (rapeseed Meal), LSP (lime stone powder) and DCP (di-calcium phosphate).

Enzyme analysis

The enzyme preparation was found to contain a-amylase 2000 ± 51 mIU/g; β-glucanase 150 ± 25 mIU/g; xylanase 3000 ± 48 mIU/g; carboxymethyl cellulase 40 ± 12.5 mIU/g; pectinase 150 ± 48 mIU/g; proteinase 600 ± 52 mIU/g; α-galactosidase 250 ± 38 mIU/g; β- galactosidase 200 ± 21 mIU/g; lipase 400 ± 45 mIU/g and phytase 50± 4.8 mIU/g. The activities (mIU/kg) of different enzymes in fermented bran were β- glucosidase 17566; β- D-xylosidase 49233; xylanase 1101; carboxymethyl cellulase 953; FTPase (filter paper degrading activity) 193 and α-amylase 2889.

4.2.2 Metabolizable energy bio-assay

Metabolizable energy bio-assay of WB

The data pertaining to feed consumption, crude protein and energy intake as well as excreta dry matter by cockerels have been presented in Table 4.2.2 and the analyses of variance of the same in Table 4.2.2a (given as Annexure). Whereas, the data on AMEn (kcal/kg), dry matter metabolizability (DMM, %) and gross energy metabolizability (GEM, %) of diets, WB and their different fermented forms fed to cockerels have been presented in Table 4.2.3 and the analyses of variance of the same in Tables 4.2.3a (given as Annexure), respectively. Intake of feed, crude protein and energy by cockerels and excreta dry matter did not differ ($P>0.05$) in different test diets. The diets having FWB (60:40) recorded significantly lower ($P<0.01$) protein intake as compared to raw and other fermented types of WB. However, the feed intake was almost similar for all fermented forms of WB. The diets having

raw WB recorded higher protein and feed intake whereas it recorded lower energy intake as compared to all fermented forms. The dry matter and energy metabolizability was significantly higher ($P<0.01$) for different test diets. Higher GEM was observed for FWB (70:30) followed by FWB (60:40) and FWB (50:50) whereas there was similar trend for all individual fermented forms of WB. The estimated AMEn values for raw WB, FWB (70:30), FWB (60:40) and FWB (50:50) were 1052, 1280, 1240 and 1262 kcal/kg, respectively. The fermentation of wheat bran improved AMEn values of FWB (70:30), FWB (60:40) and FWB (50:50) by 21.67, 17.87 and 19.96 % over their raw counterpart, respectively. AME^{mc} (mineral corrected) and AMEn (nitrogen corrected) of test diets and individual WB types differed ($P<0.01$) significantly due to fermentation technique.

The ME (metabolizable energy) or AME (apparent metabolizable energy) values have been used to express available energy value of WB and their fermented forms. The most important enzymes present in WB are glucoamylase and proteases and fermentation enzymes such as neutral protease, maltase, diastase, etc. Fermentation is an anaerobic process in which these various intrinsic enzymes of WB are activated resulting into release of nutrients and consequently their increased availability. The results indicated that the dry matter and energy metabolizability was superior ($P<0.05$) for FWB. The estimated metabolizable energy (AMEn) values for FWB were significantly higher than their corresponding raw counterparts (Fig. 4.2.1).

Table 4.2.2: Intake of feed, CP and AMEn (g or kcal/b/d) & excreta DM (%) in cockerels for different test diets of wheat bran (WB)

Interaction		Feed intake	CP intake	AMEn intake	Excreta DM
Reference diet*		89.83	13.46	275.31	24.94
WB (Raw)	200 g/kg	91.13	14.27	243.95	35.53
	400g/kg	91.01	17.90	206.33	37.67
FWB (70:30)	200 g/kg	90.89	13.27	247.23	34.64
	400g/kg	90.89	17.85	214.63	30.08
FWB (60:40)	200 g/kg	90.90	12.95	246.16	33.81
	400g/kg	90.90	16.83	213.89	33.92
FWB (50:50)	200 g/kg	90.88	13.53	246.54	38.05
	400g/kg	90.88	17.56	214.59	34.32
Substrate types					
WB (Raw)		91.07^{p}	16.09^{p}	225.14^{q}	36.60^{p}
FWB (70:30)		90.89^{q}	15.56^{q}	230.93^{p}	32.36^{r}
FWB (60:40)		90.90^{q}	14.89^{r}	230.02^{p}	33.87^{q}
FWB (50:50)		90.88^{q}	15.55^{q}	230.56^{p}	36.19^{p}
Inclusion levels					
200 g/kg		90.95^{w}	13.51^{x}	245.97^{w}	35.51^{w}
400 g/kg		90.92^{x}	17.54^{w}	212.36^{x}	34.00^{x}
SEM		0.000	0.000	0.452	0.075
Probability					
WB types		P<0.01	P<0.01	P<0.01	P<0.01
Inclusion levels		P<0.01	P<0.01	P<0.01	P<0.01
Interaction		NS	NS	NS	NS

(^{pqr}Wheat bran types) and (^{wx}Inclusion levels). Values bearing different superscripts within a column differ significantly.

WB- Wheat bran and FWB- Fermented wheat bran.

NS- Non-significant (P>0.05).

*Values not included in statistical analyses.

The fermentation of wheat bran improved AMEn values of FWB (70:30), FWB (60:40) and FWB (50:50) by 21.67, 17.87 and 19.96 % over raw counterpart, respectively. However, AMEn (nitrogen corrected) did not differ (P>0.05) significantly due to WB to moisture ratio.

The estimated value of AMEn of raw wheat bran was 1052 kcal/kg. The value was similar to earlier values of 1020 kcal/ kg and 1007 kcal/ kg on DM basis for adult male chicken and guinea fowl, respectively (Mandal and Pathak, 1996).

Table 4.2.3: AMEn (kcal/kg), dry matter (DMM, %) and gross energy metabolisability (GEM, %) of diets and wheat bran (WB) fed to cockerels

Interaction		Diets			DB	
		AMEn^{mc}	GEM	DMM	AMEn	DMM
Reference diet*		3116	73.91	72.05	-	-
WB (Raw)	200 g/kg	2704	58.55^{d}	61.01^{d}	1054	61.40^{d}
	400g/kg	2290	56.70^{e}	58.61^{e}	1051	59.07^{e}
FWB (70:30)	200 g/kg	2747	64.33^{a}	61.89^{c}	1272	62.27^{c}
	400g/kg	2385	63.35^{b}	66.91^{a}	1289	67.24^{a}
FWB (60:40)	200 g/kg	2735	63.88^{ab}	62.81^{b}	1212	63.17^{b}
	400g/kg	2377	57.09^{e}	62.68^{b}	1267	63.05^{b}
FWB (50:50)	200 g/kg	2735	63.88^{ab}	62.81^{b}	1236	58.54^{e}
	400g/kg	2385	60.10^{c}	62.23^{bc}	1288	62.61^{bc}
WB types						
WB (Raw)		2497^{q}	57.62^{s}	59.81^{r}	1052^{q}	60.23^{r}
FWB (70:30)		2566^{p}	63.84^{p}	64.40^{p}	1280^{p}	64.75^{p}
FWB (60:40)		2556^{p}	60.49^{r}	62.74^{q}	1240^{p}	63.11^{q}
FWB (50:50)		2560^{p}	61.99^{q}	62.52^{q}	1262^{p}	60.57^{r}
Inclusion levels						
200 g/kg		2730^{w}	62.66^{w}	62.13^{x}	1193	61.34^{x}
400 g/kg		2359^{x}	59.31^{x}	62.61^{w}	1224	62.99^{w}
SEM		4.869	0.106	0.083	19.003	0.081
Probability						
WB types		P<0.01	P<0.01	P<0.01	P<0.01	P<0.01
Inclusion levels		P<0.01	P<0.01	P<0.01	NS	P<0.01
Interaction		NS	P<0.01	P<0.01	NS	P<0.01

(^{abcde}Interaction), (^{pqrs}Wheat bran types) and (^{wx}Inclusion levels). Values bearing different superscripts within a column differ significantly.
^{mc}Mineral corrected, WB- Wheat bran and FWB- Fermented wheat bran.
NS- Non-significant (P>0.05).
*Values not included in statistical analyses.

Fermentation improved the ME value of wheat bran, which can be attributed to removal or inactivation of toxic substances. Fermentation of feedstuffs resulted in breakdown of cellular carbohydrates and/or alteration in the structure of intracellular starch (Fry *et al.*, 1958). Gohl and Thombe (1976) reported improvement in nutritive value of barley on reconstitution, which may be attributed to reduction in β-glucan content through activation of endogenous enzymes. Higher energy metabolizability was observed for FWB (70:30) followed by FWB (50:50) and FWB (60:40) and it was lower for their raw counterparts (Table 4.2.3). However, the effect of processing technique depends upon the inherent characteristics of the bran processed. Highly significant ($P<0.01$) interaction existed between WB and their fermented forms for dry matter metabolizability and AMEn values. The lower AMEn values of raw WB as compared to their fermented forms might be attributed to the presence of arabinoxylan, β-glancans (NSPs) and lower available carbohydrate contents as estimated in the present study. The available carbohydrates were less and the fiber content was more in raw than FWB, which might be the reasons for lower AMEn values in this raw bran. ME value of WB which was in range of 1110 - 1300 kcal/kg (Miles and Nelson, 1974; Scott, 1982 and NRC, 1994). These variations were mainly due to different cultivars tested and differences in their fiber content, the method used for estimation, age of chicken, varietal differences and phytase activities etc. (Steiner *et al.* 2007). There was a highly significant negative correlation between TA content and ME because the digestibility decreased with increasing TA content (Gous *et al.*, 1982; Janssen and Carre, 1985). The wheat starch has a poor availability in broiler chickens and this is related to a starch load in the gizzard function. Wheat bran contains arabinoxylan and

β-glancans in aleurone layers and arabinoxylans and cellulase in cell wall of pericarp and testa (Henry, 1985). Studies indicated that the viscous, water-soluble β-glucans and arabinoxylans were mainly responsible for the anti-nutritive properties of WB in broilers (Annison *et al.*, 1996). Cell wall NSP compounds were at appreciable levels of 50-80 g/kg. The soluble NSP content lowered the ME of certain wheat grains for poultry species (Annison, 1991).

Metabolizable energy bio-assay of DORB

For DORB, the data pertaining to feed consumption, crude protein and energy intake as well as excreta dry matter by cockerels have been presented in Table 4.2.4 and the analyses of variance of the same in Table 4.2.4a (given as Annexure). Whereas, the data on AMEn (kcal/kg), dry matter metabolizability (DMM, %) and gross energy metabolizability (GEM, %) of diets, DORB and their different fermented forms fed to cockerels have been presented in Table 4.2.5 and the analyses of variance of the same in Tables 4.2.5a (given as Annexure).

Intake of feed, crude protein and energy by cockerels and excreta dry matter did not differ ($P>0.05$) in different test diets. The diets having FDORB (70:30) recorded lower protein intake as compared to raw and other fermented forms of DORB. However, the feed intake was almost similar for all fermented forms of DORB. The diets having FDORB (60:40) recorded higher protein intake whereas the diets having WB (raw) was recorded lower energy intake as compared to all fermented forms. The dry matter and energy metabolizability was significantly higher ($P<0.01$) for different test diets except test diet containing FDORB (70:30).

Table 4.2.4: Intake of feed, CP and AMEn (g or kcal/b/d) & excreta DM (%) in cockerels for different test diets of de-oiled rice bran (DORB)

Interaction		Feed intake	CP intake	AMEn intake	Excreta DM
Reference diet*		89.83	13.46	275.31	24.94
DORB (Raw)	200 g/kg	90.33	14.02	258.74	30.27
	400g/kg	92.06	18.01	245.16	31.98
FDORB (70:30)	200 g/kg	90.90	13.25	262.64	38.47
	400g/kg	90.90	17.81	244.84	26.66
FDORB (60:40)	200 g/kg	90.87	14.20	265.64	31.21
	400g/kg	90.87	18.09	243.59	28.11
FDORB (50:50)	200 g/kg	90.87	14.29	265.08	30.74
	400g/kg	90.87	17.93	246.98	28.11
DORB types					
DORB (Raw)		91.20	16.02^{p}	251.95	31.12^{pq}
FDORB (70:30)		90.90	15.53^{q}	253.74	32.56^{p}
FDORB (60:40)		90.87	16.15^{p}	254.62	29.66^{q}
FDORB (50:50)		90.87	16.11^{p}	256.03	29.43^{q}
Inclusion levels					
200 g/kg		90.74^{x}	13.94^{x}	263.02^{w}	32.67^{w}
400 g/kg		91.18^{w}	17.96^{w}	245.14^{x}	28.72^{x}
SEM		0.057	0.026	0.700	0.384
Probability					
DORB types		NS	P<0.01	NS	P<0.05
Inclusion levels		P<0.01	P<0.01	P<0.01	P<0.01
Interaction		NS	NS	NS	NS

(^{pq}De-oiled rice bran types) and (^{wx}Inclusion levels). Values bearing different superscripts within a column differ significantly.
DORB- De-oiled rice bran and FDORB- Fermented de-oiled rice bran.
NS- Non-significant (P>0.05).
*Values not included in statistical analyses.

However, higher GEM was observed for FDORB (60:40) followed by DORB (raw) and FWB (50:50) whereas it was similar trend for all individual fermented forms of DORB. The estimated AMEn values for raw DORB, FDORB (70:30), FDORB (60:40) and FDORB (50:50) were 2025, 2127, 2197 and 2228 kcal/kg, respectively (Fig. 4.2.2). Fermentation of DORB after soaking it with different proportion of water viz. 70:30, 60:40 or 50:50 improved its AMEn value by 5.04,

8.49 and 10.02%, respectively. AMEn (nitrogen corrected) did not differ significantly due to fermentation technique (P>0.05). The present estimation of ME value corroborated with the findings of the earlier value of 2069 and 1998 kcal/kg in chicken and guinea fowl, respectively (Mandal and Pathak 1996).

Table 4.2.5: AMEn (kcal/kg), dry matter (DMM, %) and gross energy metabolizability (GEM, %) of diets and de-oiled rice bran (DORB) fed to cockerels

Interaction		Diets			DORB	
		AMEn^{mc}	GEM	DMM	AMEn	DMM
Reference diet*		3116	73.91	72.05	-	-
DORB (Raw)	200 g/kg	2893	69.64^{ab}	66.50^{c}	2001	67.08^{c}
	400g/kg	2690	67.73^{cd}	65.26^{e}	2050	65.21^{e}
FDORB (70:30)	200 g/kg	2918	63.76^{e}	57.68^{f}	2127	58.10^{f}
	400g/kg	2720	70.67^{a}	70.67^{a}	2127	70.96^{a}
FDORB (60:40)	200 g/kg	2953	69.37^{ab}	65.66^{de}	2299	66.00^{d}
	400g/kg	2708	68.32^{bc}	69.07^{b}	2095	69.37^{b}
FDORB (50:50)	200 g/kg	2946	69.64^{ab}	66.18^{cd}	2267	66.51^{cd}
	400g/kg	2745	66.64^{d}	69.06^{b}	2189	69.37^{b}
DORB types						
DORB (Raw)		2791	68.68^{p}	65.88^{q}	2025	66.14^{q}
FDORB (70:30)		2819	67.21^{q}	64.18^{r}	2127	64.53^{r}
FDORB (60:40)		2830	68.84^{p}	67.36^{p}	2197	67.69^{p}
FDORB (50:50)		2846	68.14^{pq}	67.62^{p}	2228	67.94^{p}
Inclusion levels						
200 g/kg		2927^{w}	68.10^{x}	64.00^{x}	2173	64.42^{x}
400 g/kg		2716^{x}	68.34^{w}	68.52^{w}	2115	68.73^{w}
SEM		7.631	0.183	0.096	31.028	0.095
Probability						
DORB types		NS	P< 0.05	P<0.01	NS	P<0.01
Inclusion levels		P<0.01	NS	P<0.01	NS	P<0.01
Interaction		NS	P<0.01	P<0.01	NS	P<0.01

(^{abcdef}Interaction), (^{pqr}De-oiled rice bran types) and (^{wx}Inclusion levels). Values bearing different superscripts within a column differ significantly.
^{mc}Mineral corrected, DORB- De-oiled rice bran and FDORB- Fermented de-oiled rice bran.
NS- Non-significant (P>0.05).
*Values not included in statistical analyses.

Fig 4.2.1: **Apparent metabolizable energy (N-corrected) values (kcal/kg) of wheat bran (WB) and their fermented forms (FWB)**

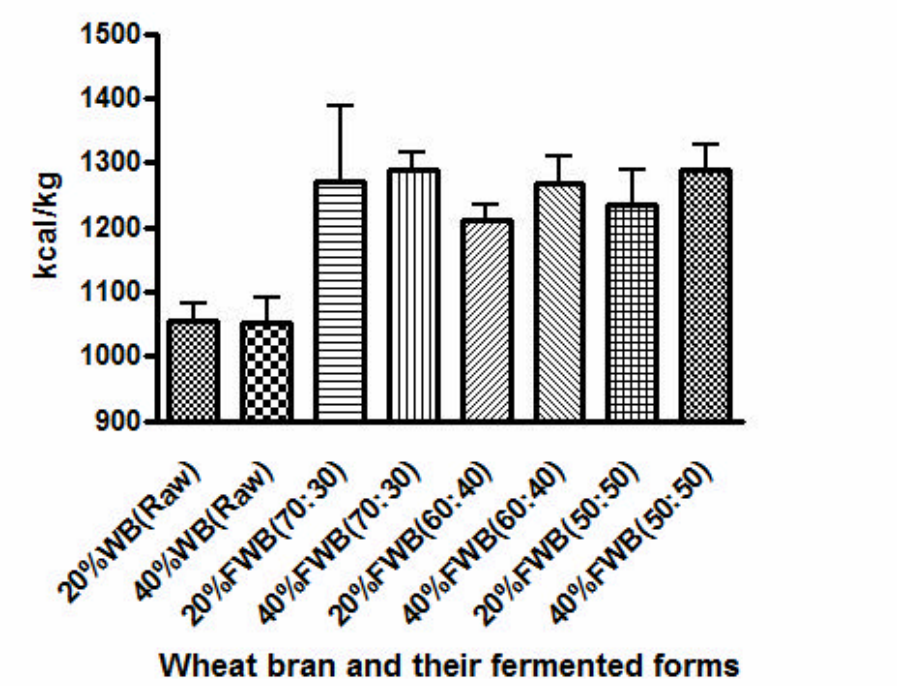
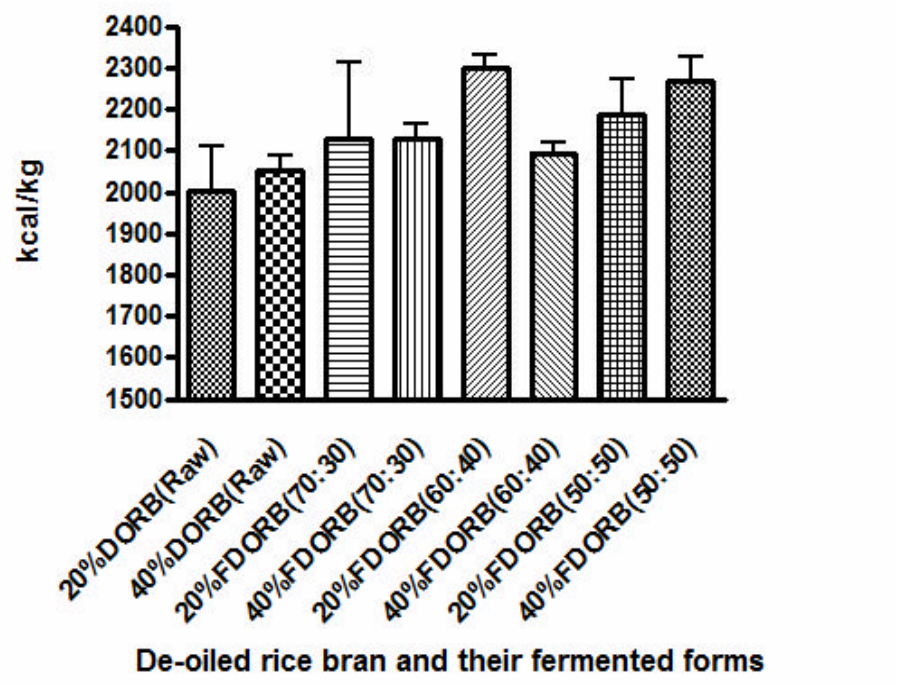


Fig 4.2.2: **Apparent metabolizable energy (N-corrected) values (kcal/kg) of de-oiled rice bran (DORB) and their fermented forms (FDORB)**



The ME (metabolizable energy) or AME (apparent metabolizable energy) values have been used to express available energy value of DORB and other cereal byproducts. The most important enzymes present in DORB are amylase, proteases, lipases, and fermentation enzymes such as α-amylase, acid proteases, lipases, etc. Fermentation is an anaerobic process in which these various intrinsic enzymes (NSP enzymes and phytase) of bran origin are activated resulting into release of nutrients and consequently their increased availability. The possible mechanism of improvement has already been discussed earlier.

4.2.3 *In vitro* pepsin-pancreatin digestibility (IVPPD)

The data on IVPPD of fermented and unprocessed WB and DORB have been presented in Table 4.2.6 and the analyses of variance of the same in Table 4.2.6a (given as Annexure). Fermentation of the WB increased ($P<0.05$) IVPPD of FWB (70:30), FWB (60:40) and FWB (50:50) by 9.09, 3.98 and 5.12% over their raw counterpart, respectively (Fig. 4.2.3). When different bran to water ratio for fermentation was compared maximum improvement was observed as fermented with less water.

Fermentation of the DORB increased ($P<0.01$) IVPPD of FDORB (70:30), FDORB (60:40) and FDORB (50:50) by 7.54, 8.09 and 9.78% over the unprocessed DORB, respectively (Fig. 4.2.4).

Various processing treatments are known to improve its digestibility and nutritive value (Alka-Sharma and Kapoor, 1996). Fermentation (Chavan *et al.*, 1988; Usha *et al.*, 1996) and sprouting (Chavan and Kadam, 1989) have been also reported to increase the protein digestibility of millet.

The results obtained in this study agree with those reported by Di Lena *et al*. (1997) who revealed that a significant ($P<0.05$)

increment in protein digestibility of FWB through solid substrate fermentation by using *Lentinula edode*, and the protein digestibility of FWB increased gradually from 62 to 82%. In present study, IVPPD of FWB was increased from 69.29 to 75.59%. Laufenberg *et al.* (2003); Anupama and Ravindra (2000) and Moraes (1999) demonstrated that some fungal species were able to increase the protein level in agro-industries by-products. The same observation along with significant increase in protein digestibility was reported on fermentation of WB by *A. oryzae* (Silveira and Badiale-Furlong, 2009).

Moreover, El Hag *et al.* (2002) also found that fermentation of millet seeds improved its *in vitro* protein digestibility. Manwar and Mandal (2008) observed concurrent results; wherein, reconstitution of the grain increased ($P<0.01$) the IVPPD. The IVPPD of the treated grains after reconstitution with enzymes was greatly improved. The increase in protein digestibility content of the reconstituted grains was attributed to quantitative reduction in antinutritional factors (tannin, polyphenols and phytic acid), which are known to interact with proteins to form complexes resulting in reduced digestibility (Manwar and Mandal, 2008). Similarly, El Hag *et al.* (2002) deduced that enhanced proteolytic activity during fermentation might be generally associated with improved protein digestibility, which increased amino nitrogen by partial breakdown of protein peptides and amino acid. Conversely, there was significant reduction in protein digestibility of WB on fermentation with *Rhizopus* sp. (Silveira *et al.*, 2003; Silveira and Badiale-Furlong, 2009).

The results of present study showed that IVPPD of FDORB was increase from 69.93 to 76.77%. The findings were in accordance with Di Lena *et al.* (1997) who reported a significant ($P<0.05$) surge in

protein digestibility of FDORB through solid substrate fermentation of DORB using *Lentinula edode* linearly from 62 to 82%. However, there was significant reduction in protein digestibility of DORB, when fermented with *Rhizopus* sp. Also, fermentation using *A. oryzae* did not lead to significant increase in protein digestibility of the FDORB, (Silveira and Badiale-Furlong, 2009). According to Sze-Tao and Sathe (2000), there was positive correlation between the protein digestibility and solubility in aqueous systems. However, the less IVPPD of untreated bran in present study might be attributed to the antinutritional factors such as β-glucans and arabinoxylans in aleurone layers of WB (Annison *et al.*, 1996) and phytotoxicins in DORB. The presence of various antinutritional factors, poor digestibility of the protein and carbohydrates greatly affected its utilization in birds. The per cent increase in protein digestibility content of the fermented bran might be due to quantitative reduction in antinutritional factor, which are known to interact with proteins to form complexes resulting in reduced digestibility. The variations between the cereals bran response to different processing treatments might be attributed to the nature and type of proteins of cereal bran. Various processing treatments are known to improve its digestibility and nutritive value of cereal and their byproducts (Alka-Sharma and Kapoor, 1996). Fermentation (Chavan *et al.*, 1988; Usha *et al.*, 1996) and sprouting (Chavan and Kadam, 1989) have been also reported to increase the protein digestibility of millet. The increased digestibility may have been directly related to the hydrolysis of phytic acid, which was previously reported to be a factor adversely affecting protein digestibility, as well as the enzymatic digestion of the plant cell walls. By the time phytate hydrolysis was completed, protein digestibility was still gradually increasing, probably an effect of cell wall degrading activities (Di Lena *et al.*, 1997).

It may be inferred that solid substrate fermentation proves to be effective in increasing the IVPPD of the WB and DORB.

4.2.4 Available carbohydrates (ACHO)

Available carbohydrate content of fermented and unprocessed WB or DORB have been presented in Table 4.2.6 and the analyses of variance of the same in Table 4.2.6a (given as Annexure).

There was significant difference in ACHO content in raw or fermented WB ($P<0.01$). Fermentation of WB reduced ($P<0.01$) the ACHO content in FWB (70:30), FWB (60:40) and FWB (50:50) by 36.06, 24.06 and 24.29% over the raw WB, respectively (Fig. 4.2.3).

Fermentation of the DORB decreased ($P<0.01$) ACHO content in FDORB (70:30), FDORB (60:40) and FDORB (50:50) by 19.37, 9.61 and 32.87% over the unprocessed DORB, respectively (Fig. 4.2.4).

Carbohydrates are quantitatively the most important constituents, forming about 66 and 52% of the total dry matter of WB and DORB, respectively (Silveira and Badiale-Furlong, 2009 and Silva *et al.*, 2001). The carbohydrates present in cereal grains include more than 90% of starch. The remaining portion is cellulose, hemicellulose, pentosans, dextrins and sugars. Cereal starches are more or less similar in composition, having 74-79% amylopectin, 25-30% amylose, and 1% lipid. The available carbohydrate (ACHO) content in grains, however, was not significantly affected due to reconstitution with or without enzymes (Manwar and Mandal, 2008). Moreover, there was significant difference in ACHO content in different cereal grains (Manwar and Mandal, 2008).

There was significant decrease of ACHO on fermentation, which may be attributed to growth of *Aspergillus* as the fungi utilizes available carbohydrates first for their growth.

4.2.5 Available phosphorus

The percent total phosphorus, phytate P and non- phytate P and their proportion of total phosphorus have been given in Table 4.2.6 and the analyses of variance of the same in Table 4.2.6a (given as Annexure).

WB contained relatively higher amount of total phosphorus. Percent phytate P decreased significantly due to fermentation up to the extent of 11 to 19% for all the fermented forms of WB. Thus, the available phosphorus (% total phosphorus) contents of FWB (70:30), FWB (60:40) and FWB (50:50) were 62.35, 59.98 and 54.83%, respectively (Fig. 4.2.3).

DORB had relatively higher amount of total phosphorus content. Phytate P decreased significantly ($P<0.01$) due to fermentation up to the extent of 30 to 42% for all the fermented forms of DORB. Thus, the available phosphorus (% total phosphorus) contents of FDORB (50:50), FDORB (60:40) and FWB (70:30) were 45.88, 45.18 and 44.13%, respectively (Fig. 4.2.4).

About 85 per cent of the total phosphorus found in feedstuffs of vegetable origin, particularly the cereals, cereal by-products and oil cakes is present in form of phytic acid (inositol 1, 2, 3, 4, 5, 6-hexa-phosphate). Phytate reduces the bioavailability of minerals, and the solubility, functionality and digestibility of proteins and carbohydrates. Phytic acid has strong chelating potential and forms a variety of complexes with cations and proteins, rendering these nutrients biologically unavailable. Theoretically, when phytic acid is hydrolysed by microbial phytase, all minerals bound to it are released. Under most dietary conditions, the phytate phosphorus is poorly utilized by monogastrics including poultry and consequently, excreted

via the feces. Though brans are rich source of phosphorus, its bioavailability from bran is only about 30%. The hydrolysis and absorption of phytate phosphorus by monogastric animals are complex processes that are affected by certain factors namely-dietary calcium and inorganic phosphorus (or available phosphorus) levels, vitamin D_3, age and type (genotype) of birds, types of dietary ingredients, sources of fiber in diet and feed processing etc. The values reported in the literature on the availability in most of the single and many feed mixtures are variable. Although P has more known functions in the body than any other mineral nutrient (Lynch and Caffrey, 1997), it is one of the main polluting nutrients from animal agriculture. In view of such undesirable effects of phytic acid, it is preferred to either remove it altogether or reduce its amount in poultry feed or ingredients. Efforts have been made to either eliminate or reduce phytic acid content in plant feedstuffs through chemical methods, solid-state fermentation technology, and autolysis or by the use of phytase enzyme in diet. Fermentation of WB or cereal-byproducts reduces phytate content *via* the action of phytases that catalyze conversion of phytate to inorganic orthophosphate and a series of myo-inositols, lower phosphoric esters of phytate. A 3-phytase appears to be characteristic of microorganisms, while a 6-phytase is found in WB and other plant seeds (Reddy and Pierson, 1994). The cereal bran exhibits relatively high total phosphorus content. Total P concentration in cereal grains and by-products ranged from 0.12 to 1.57%. Phytic P concentrations were greater in cereals and oil seed by-products (0.24-1.13%) than in grains (0.08-0.49%). Phytic P, as percent of total P, was reaching values over 70% for wheat bran and rice polishing (Godoy *et al.*, 2005). Salts of phytic acid are deposited in the aleurone, scutellum, cotyledon, and endosperm during seed formation (Bergman *et al.*, 2000). Phytates in cereals or bran are not

uniformly distributed within the kernel but found in abundance in aleurone layer of the grains and bran (Reddy *et al.*, 1989 and Ravindran *et al.*, 1999). Phytic P, as percent of total P, was 73% (70-76%) and 84% (80-87%) for WB and DORB, respectively (Tyagi *et al.*, 1998a), whereas WB and RP contains little phytase activity (Godoy *et al.*, 2005). In general, digestibility of P in cereal grains and their by-products is lower than the digestibility of P in commonly used inorganic supplements.

The fermentation of WB and DORB greatly reduced ($P<0.01$) the phytate phosphorus content of all fermented forms (Fig. 4.2.3 and 4.2.4, respectively). The phytate P present in the wheat was hydrolyzed into inorganic P to an extent of 27.6% on soaking of ground wheat in water for 18 h (Tyagi *et al.*, 1997). The difference in the rate of hydrolysis of phytate P by water soaking of WB and/or DORB during fermentation and in present experiment compared to the other reports can, therefore, be attributed to some of the factors such as the endogenous phytase activity of WB and RP. It has been reported the endogenous phytase activity in WB and RP had > 100 units/kg depending not only upon cultivar, soil chemistry, and age of wheat and rice and/ or drying and storage conditions but also upon pH and temperature of the medium and time of soaking (Eeckhout and De Paepe, 1994, Godoy *et al.*, 2005). Di-Lena *et al.* (1997) noted a significant ($P<0.05$) reduction in the phytic acid content of WB as a result of solid state fermentation of WB, it was reduced by 60% after a week of incubation period. Phytate reduction by phytases produced during the solid state fermentation of different microorganism has been reported in various cereal-byproducts including WB and cottonseed flour (Segueilha *et al.*, 1993), soybean and cottonseed meals (Han and Wilfred, 1988) and canola meal (Nair *et al.*, 1991).

Fig 4.2.3: Effects of fermentation of wheat bran (WB) on availability of certain nutrients viz. IVPPD, ACHOS, ADF and Avl. P (%)

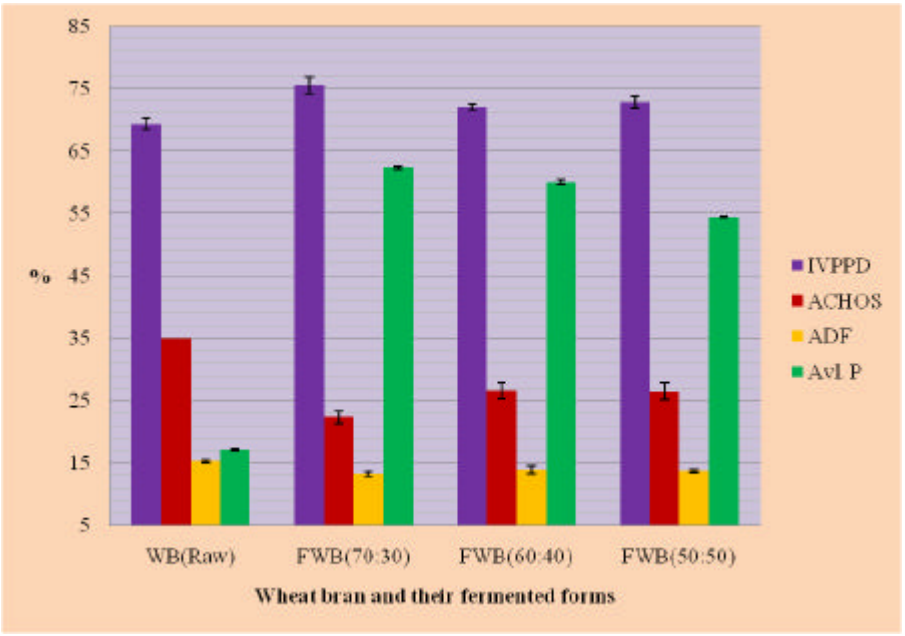
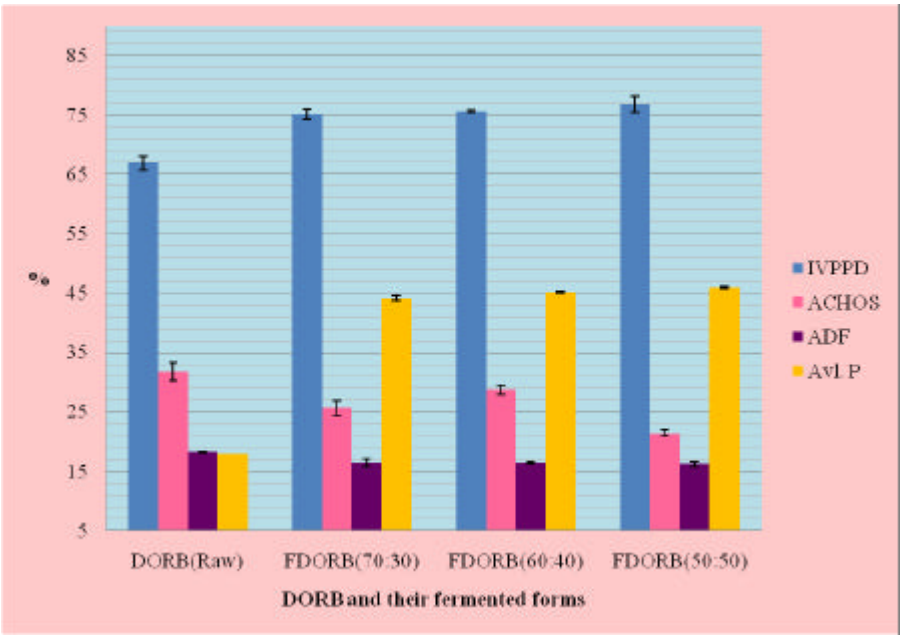


Fig 4.2.4: Effects of fermentation of de-oiled rice bran (DORB) on availability of certain nutrients viz. IVPPD, ACHOS, ADF and Avl. P (%)



The results showed that phytate were decreased significantly due to fermentation as also observed by earlier workers when grains were reconstituted with or without enzymes (Manwar and Mandal, 2008). Therefore, fermentation would be an effective process for increasing availability of P.

4.2.6 Acid detergent fiber (ADF)

The results of ADF analysis of WB and DORB are given in Table 4.2.6 and the analyses of variance of the same in Table 4.2.6a (given as Annexure). The data revealed a great variability in the ADF contents. The ADF content was maximum in raw WB and DORB, which reduced significantly ($P<0.01$) in their respective fermented forms. As moisture level in WB substrate decreased, there was reduction the ADF content of FWB. When FWB soaked with water at the ratio of 70:30 (w/v), the ADF content was reduced by 13.01% over their raw counterpart (Fig. 4.2.3).

However, the ADF content was comparable amongst the different fermented forms of DORB. Fermentations of DORB tended to decrease ($P<0.05$) the ADF content in their different forms. When FDORB soaked with water at the ratio of 50:50 (w/v), the ADF content reduced by 10.88% over unprocessed DORB (Fig. 4.2.4).

It can be seen that there was a great variability in ADF contents of WB and DORB (Fig. 4.2.3 and 4.2.4, respectively). The major sugars identified in NSP in raw WB, DORB and their corresponding fermented forms were arabinose, xylose, galactose and glucose. Mannose and rhamnose are present as minor constituents. Raw WB polysaccharides mainly consisted of arabinose (27%), xylose (39%), galactose (2%) and glucose (30%) with traces of mannose (Hegade *et*

al., 2006). As against the raw WB, in FWB soaked with water at the ratio 50:50 (w/v), there was 9- and 19-fold decrease in the arabinose and xylose contents, respectively, in a 96 h growth period of *A. niger.* Similarly a marginal decrease was observed in the rhamnose/fucose content. The substantial decrease in the arabinose and xylose content indicates the degradation of arabinoxylan backbone of the WB polysaccharide (Hegde *et al.,* 2006). Also, earlier work pertaining to the various levels of cell wall degrading enzymes, wherein, xylanase activity is found to be higher, indicating extensive degradation of the xylan backbone (Kavitha *et al.*, 2004).

Raw DORB consisted of arabinose 9%, xylose 27%, galactose 30% and glucose 32%. More than 4-fold decrease was observed in the arabinose content in the FDORB soaked with water ratio 50:50 (w/v), whereas xylose was degraded by 13-fold (Hegde *et al.,* 2006). Previous studies revealed extensive degradation of arabinoxylans rather than hexosans during the growth of the fungus, which can be correlated with higher activity of xylanase (Kavitha *et al.,* 2004). Manwar and Mandal (2008) reported that the reconstitution with or without enzymes reduced the ADF contents in the cereal grains. Conversely, total and insoluble dietary fiber in bran appeared to increase significantly ($P<0.05$) during solid substrate fermentation. This could be due to the increase in indigestible chitin, the fungal cell wall aminopolysaccharide acting as insoluble dietary fibre (Di-Lena *et al.*, 1997). There was a significant amount of glucose in untreated bran as well as in all fermented forms of bran, which originated from cellulose (Bonnin *et al.*, 2002) and β-D glucan (Brillouet, J *et al.*, 1982) and starch.

Table 4.2.6: Effects of fermentation of wheat bran (WB) and de-oiled rice bran (DORB) on availability of certain nutrients

Parameters	IVPPD (%)	ACHOS (%)	Phytate P (%)	Phytate P (%, TP)	NPP (%)	NPP (%, TP)	ADF (%, DM)
Wheat bran (WB) types							
WB (Raw)	69.29^{c}	35.00^{a}	1.01^{a}	82.89^{a}	0.21^{d}	17.11^{d}	15.22^{a}
FWB (70:30)	75.59^{a}	22.37^{c}	0.90^{b}	62.35^{b}	0.55^{c}	62.35^{a}	13.24^{b}
FWB (60:40)	72.05^{bc}	26.58^{b}	0.89^{c}	59.98^{c}	0.59^{b}	59.98^{b}	13.87^{b}
FWB (50:50)	72.84^{ab}	26.50^{b}	0.82^{d}	54.83^{d}	0.67^{a}	54.83^{c}	13.67^{b}
SEM	0.792	1.455	0.021	3.222	0.053	5.539	0.281
Stast.	P<0.05	P<0.01	P<0.01	P<0.01	P<0.01	P<0.01	P<0.05
De-oiled rice bran (DORB) types							
DORB (Raw)	69.93^{q}	31.85^{p}	1.25^{p}	82.00^{p}	0.27^{s}	18.00^{r}	18.20^{p}
FDORB (70:30)	75.20^{p}	25.68^{q}	0.72^{s}	55.87^{q}	0.56^{r}	44.13^{q}	16.39^{q}
FDORB (60:40)	75.59^{p}	28.79^{pq}	0.87^{q}	54.82^{r}	0.72^{p}	45.18^{p}	16.46^{q}
FDORB (50:50)	76.77^{p}	21.38^{r}	0.74^{r}	54.12^{r}	0.62^{q}	45.88^{p}	16.22^{q}
SEM	1.254	1.258	0.064	3.540	0.050	3.540	0.289
Stast.	P<0.01	P<0.01	P<0.01	P<0.01	P<0.01	P<0.01	P<0.05

(^{abcd}Wheat bran types) and (^{pqr}De-oiled rice bran types). Values bearing different superscripts within a column differ significantly.
NS- Non-significant (P>0.05). TP- Total P. NPP - Non phytate P.
WB-Wheat bran, FWB-Fermented wheat bran, DORB-De-oiled rice bran and FDORB-Fermented de-oiled rice bran.

4.3 EXPERIMENT 3: EFFECT OF FEEDING FERMENTED WHEAT BRAN WITH OR WITHOUT ENZYME ON GROWTH AND IMMUNE RESPONSE IN BROILERS

In the present study, the efforts were made to investigate the effect of feeding diets containing fermented wheat bran with or without enzyme on growth performance, nutrient utilization, immune responsiveness, carcass traits, certain blood biochemicals, caeca microbial load and cost of feeding of broiler chickens.

4.3.1 Growth performance

The results on growth performance of broiler chicken as influenced by the feeding of fermented wheat bran, with or without

enzymes in broiler diets are presented and discussed hereunder. The growth performance of experimental broiler chicks with respect to gain in body weight, feed intake, feed conversion ratio was evaluated at weekly intervals (0-6 weeks of age) and phase wise i.e. starting phase (0-3 weeks of age), finishing phase (3-6 weeks of age) and overall phase (0-6 weeks of age).

Body weight gain, feed intake and feed conversion ratio

The data on mean body weight gain of chicks, feed intake and feed conversion ratio at different phases of growth of broilers have been presented in Table 4.3.1, Tables 4.3.2 and 4.3.3 at weekly intervals (0-6 weeks of age) and phase wise i.e. starting phase (0-3 weeks of age), finishing phase (3-6 weeks of age) and overall phase (0-6 weeks of age), respectively and the analyses of variance of the same in Tables 4.3.1a, 4.3.2a and 4.3.3a (given as Annexure), respectively.

On weekly basis, it is evident that there were significant differences in body weight gains (BWGs) of broiler chicks under different treatments of WB at included levels either 5% or 7.5% raw WB and/or FWB during 1st to 5th week of age. However, during 6th week of age, the mean BWGs were no significant differences amongst treatments. The differences in BWG during 0-5 wks of age (weekly), though differed significantly, did not show any definite trend. At 6th weeks of age, the mean BWG of the birds fed diets containing FWB at 5% inclusion level (1536 g) was numerically higher than control and other treatment groups. On cumulative analysis, BWGs of broilers had significant ($P<0.01$) differences amongst treatments during 0-3 (starting phase) weeks of age. The birds fed FWB at 5% inclusion level (D_7), raw WB at 0, 5 and 7.5 % inclusion level with enzymes (D_4, D_5 and D_6) had significantly ($P<0.01$) higher BWGs than the birds of control (D_1), birds fed raw WB

at both 5 and 7.5% inclusion level without enzyme (D_2 and D_3) and birds fed FWB at 7.5% level (D_8). However, the gains in body weight of broilers were not affected by the inclusion levels of either raw or fermented WB during 3-6 (finishing phase) weeks of age. Similarly, during 0-6 (overall phase) weeks of age, mean BWGs amongst different treatments were statistically non-significant. At 6th weeks of age, the mean BWG of the birds fed diets containing FWB at 5% inclusion level (1536 g) was numerically higher than control and other treatment groups (Fig. 4.3.1). Fermentation of WB improved weight gain in broilers as compared to untreated WB, which was comparable to control diet containing corn-soya diet. The enzyme supplementation to the WB based diets found beneficial in terms of body weight gain in broilers.

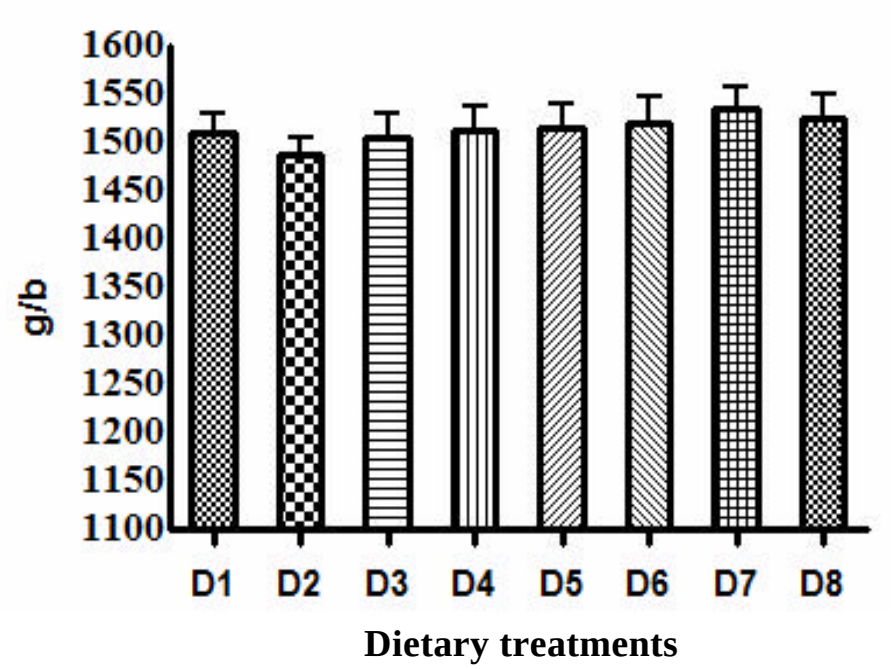
Table 4.3.1: Effect of feeding fermented wheat bran on body weight gain (g/b) of broilers at weekly intervals and different growth phases (wk)

Diet	Treatment	Body weight gain (g)								
		Weekly						Phase wise		
		1 wk	2 wk	3 wk	4 wk	5 wk	6 wk	0-3 wk	3-6 wk	0-6 wk
D_1	WB_0	83^{b}	177^{ab}	248^{bc}	298^{a}	356^{a}	347	508^{bc}	1002	1510
D_2	WB_5	95^{ab}	170^{bc}	226^{de}	282^{ab}	309^{c}	404	491^{c}	995	1486
D_3	$WB_{7.5}$	97^{ab}	180^{ab}	216^{e}	287^{ab}	331^{abc}	394	492^{c}	1012	1504
D_4	WB_0+E	94^{ab}	180^{ab}	247^{bcd}	280^{ab}	318^{c}	394	520^{abc}	992	1512
D_5	WB_5+E	106^{a}	162^{c}	253^{ab}	291^{ab}	334^{abc}	369	521^{abc}	994	1514
D_6	$WB_{7.5}$+E	104^{a}	190^{a}	235^{bcde}	274^{bc}	326^{bc}	391	529^{ab}	991	1520
D_7	FWB_5	99^{a}	185^{a}	269^{a}	258^{c}	349^{ab}	374	554^{a}	982	1536
D_8	$FWB_{7.5}$	92^{ab}	168^{bc}	230^{cde}	292^{ab}	353^{ab}	391	489^{c}	1036	1525
SEM		1.62	1.54	2.62	2.66	3.51	4.71	4.28	6.93	8.08
Stat. significance		P<0.05	P<0.01	P<0.01	P<0.01	P<0.01	NS	P<0.01	NS	NS

WB - Wheat bran, FWB – Fermented wheat bran; NS- Non-significant (P>0.05)

Data on mean feed intake of broilers obtained at weekly and cumulative intervals are presented in Table 4.3.2. Statistical analysis

Fig. 4.3.1 : Effect of feeding fermented wheat bran on body weight gain (g/b) in broilers (0-6 wk)



of the data pertaining to feed intake as influenced by different inclusion levels of either raw WB or FWB, with or without enzymes to broilers revealed that average weekly feed intake remained statistically non-significant from 1st to 2nd weeks of age and from 4th to 5th weeks of age. However, during 3rd week of feeding trail, mean feed intake were significantly ($P<0.05$) lower at 381g in D_3 than the rest of the groups except in D_2 (WB_5) and D_8 ($FWB_{7.5}$) groups. The feed intake remained statistically similar in D_1, D_4, D_5, D_6 and D_7 groups. Also, mean feed intake were significantly ($P<0.05$) lower at 656g in D_1 than the rest of the groups except in D_7 (FWB_5) and D_8 ($FWB_{7.5}$) groups. The feed intake remained statistically similar in D_2, D_3, D_4, D_5 and D_6 groups during 6th week of feeding trail. On cumulative basis, during 0-3 and 3-6 weeks of age, the feed intake was not found to be significantly affected by inclusion of raw and/or FWB, with or without enzyme supplementation. Similarly, during 0-6 weeks of overall phase, the mean feed intake amongst different treatments of broilers was statistically non significant. The feed intake of broilers in the test and control groups was comparable. When FWB inclusion levels were compared, feed intake showed a decreasing trend as the level of raw WB with or without enzyme increased in the diets. In general, feed intake decreased with the inclusion levels of WB in diet, either as raw or as fermented.

The data on feed conversion ratio (FCR) calculated as feed consumption per unit weight gain at weekly and cumulative intervals of birds as influenced by different inclusion levels of either raw WB or FWB, with or without enzymes through feed has been summarized in Table 4.3.3. Statistical analysis of the mean FCR values of birds under different treatments revealed no significant differences up to 6th weeks of age.

Table 4.3.2: Effect of feeding fermented wheat bran on feed intake (g/b) of broilers at weekly intervals and different growth phases (wk)

Diet	Treatment	Feed Intake (g)								
		Weekly						Phase wise		
		1 wk	2 wk	3 wk	4 wk	5 wk	6 wk	0-3 wk	3-6 wk	0-6 wk
D_1	WB_0	114	255	429^{ab}	544	688	656^{d}	797	1888	2685
D_2	WB_5	120	252	400^{bc}	516	606	764^{a}	772	1884	2655
D_3	$WB_{7.5}$	129	270	381^{c}	535	690	752^{ab}	779	1976	2756
D_4	WB_0+E	118	255	420^{abc}	529	645	736^{abc}	792	1910	2702
D_5	WB_5+E	126	250	458^{a}	529	657	682^{a}	833	1868	2701
D_6	$WB_{7.5}$+E	125	268	416^{abc}	517	637	733^{abc}	808	1887	2694
D_7	FWB_5	119	261	446^{ab}	472	668	667^{cd}	825	1806	2631
D_8	$FWB_{7.5}$	113	247	400^{bc}	527	677	710^{abcd}	760	1913	2673
SEM		2.84	2.36	6.16	5.84	7.84	9.61	8.60	14.16	14.08
Stat. Significance		NS	NS	P<0.05	NS	NS	P<0.05	NS	NS	NS

WB - Wheat bran, FWB – Fermented wheat bran
NS- Non-significant (P>0.05)

On cumulative analysis, the mean FCR values of broilers were not affected by the inclusion levels of either raw or fermented WB during starting phase (0-3 weeks of age) and finishing phase (3-6 weeks of age). Similarly, during 0-6 (overall phase) weeks of age, mean FCR values amongst different treatments were statistically non-significant. Almost similar trend was maintained in FCR values during 0-6 weeks of age, wherein, the mean FCR values of the birds fed diets containing FWB at 5% inclusion level (1.72) was numerically better than control and other treatment groups. However, FCR values of D_2 (WB_5) and D_3 ($WB_{7.5}$) group were comparable to the control, control with enzyme and their corresponding enzyme supplemented group. Due to fermentation of WB, improvement in FCR in broilers was evident as compared to untreated WB, which was comparable to control diet containing corn-soya diet. The enzyme supplementation to the WB based diets found beneficial in terms of FCR values in broilers.

Table 4.3.3: Effect of feeding fermented wheat bran on feed conversion ratio (FCR) of broilers at weekly intervals and different growth phases (wk)

Diet	Treatment	Feed conversion ratio (FCR)								
		Weekly						Phase wise		
		1 wk	2 wk	3 wk	4 wk	5 wk	6 wk	0-3 wk	3-6 wk	0-6 wk
D_1	WB_0	1.38	1.44	1.73	1.82	1.94	1.90	1.57	1.88	1.78
D_2	WB_5	1.27	1.48	1.77	1.83	1.96	1.89	1.57	1.90	1.79
D_3	$WB_{7.5}$	1.36	1.50	1.77	1.86	2.09	1.91	1.59	1.96	1.83
D_4	WB_0+E	1.25	1.42	1.70	1.89	2.04	1.87	1.52	1.93	1.79
D_5	WB_5+E	1.19	1.55	1.81	1.82	1.97	1.85	1.60	1.88	1.78
D_6	$WB_{7.5}$+E	1.20	1.41	1.77	1.89	1.96	1.87	1.53	1.90	1.77
D_7	FWB_5	1.22	1.41	1.66	1.83	1.92	1.79	1.49	1.84	1.72
D_8	$FWB_{7.5}$	1.25	1.48	1.75	1.81	1.92	1.82	1.56	1.85	1.75
SEM		0.022	0.015	0.018	0.014	0.025	0.015	0.011	0.012	0.010
Stat. Significance		NS	NS	NS	NS	NS	NS	NS	NS	NS

WB - Wheat bran and FWB – Fermented wheat bran
NS- Non-significant (P>0.05)

The mortality (Tabel 4.3.4) occurred during feeding trail was well within the normal range and not found to be related to the fermentation WB and their inclusion level.

Table 4.3.4: Livability of experimental birds (0-42 days)

Diet	Treatment	No. of chicks		Mortality	Livability
		Started	Survived	%	%
D_1	WB_0	32	32	0.00	100.00
D_2	WB_5	32	32	0.00	100.00
D_3	$WB_{7.5}$	32	32	0.00	100.00
D_4	WB_0+E	32	31	3.13	96.88
D_5	WB_5+E	32	31	3.13	96.88
D_6	$WB_{7.5}$+E	32	31	3.13	96.88
D_7	FWB_5	32	30	6.25	93.75
D_8	$FWB_{7.5}$	32	32	0.00	100.00

WB - Wheat bran and FWB - Fermented wheat bran

Fig. 4.3.2 : Effect of feeding fermented wheat bran on feed intake (g/b) in broilers (0-6 wk)

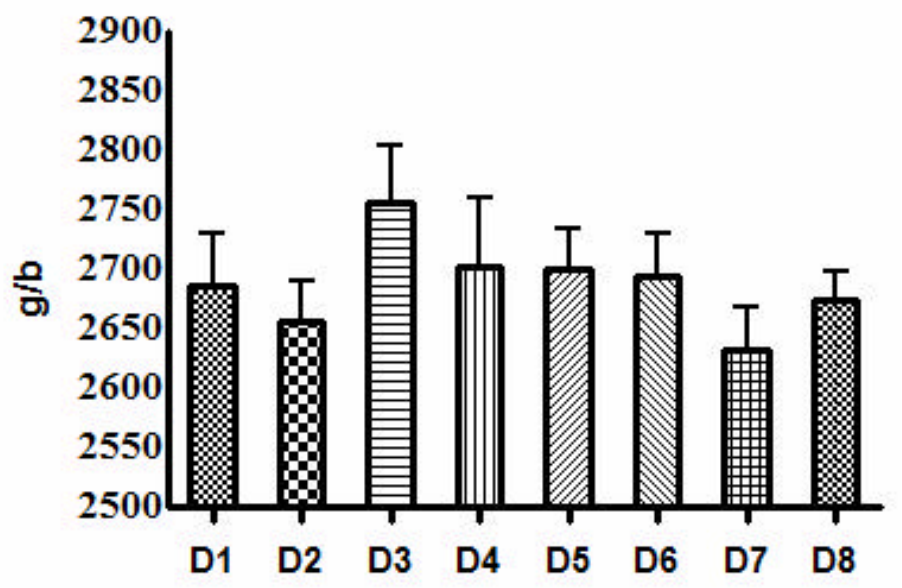
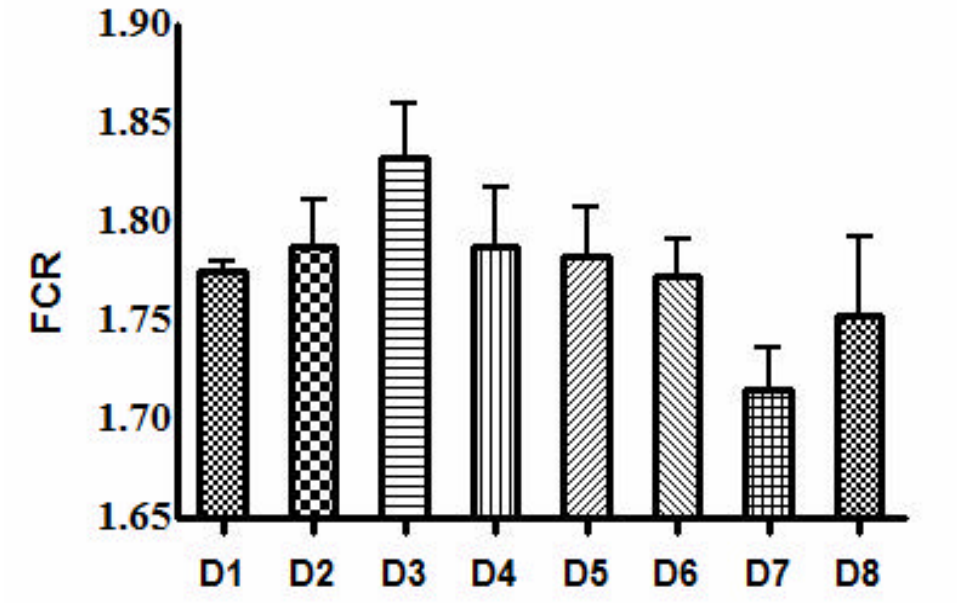


Fig. 4.3.3 : Effect of feeding fermented wheat bran on FCR of broilers (0-6 wk)



The present study has demonstrated that the chicks of D_7 group, in which FWB was included @ 5% in feed, recorded significantly higher BWGs than the groups of chicks offered control diet and raw WB, with or without enzymes in diet during starting phase (0-3 weeks of age). However, neither the fermentation nor the enzyme supplementation had any effect on gain in body weight by the experimental chicks of 3-6 weeks of age. Nevertheless, mean BWGs as influenced by different inclusion levels of either raw WB or FWB, with or without enzymes through feed to broilers were statistically non significant during overall phase (0-6 weeks of age). Similarly, feed intake and FCR values were also unaffected by the experimental diets up to 6 weeks of age. The body weight gain and feed conversion ratio were better in bird fed fermented WB in comparison to those fed untreated WB. The intake of feed in different treatments did not vary in comparison to the control group. The fermentation of WB was beneficial in improving the growth performance. Moreover, supplementation of enzymes to raw WB-based diets or addition of enzymes with 5 and 7.5% of untreated WB did not further improve the growth performance of broilers. No direct study involving feeding of fermented WB with *Aspergillus niger* to broiler chicken appear to have been carried out earlier and it seems that the present study is the first one on this aspect carried out in this laboratory. However, Darwazeh (2010) when fermented WB with rumen filtrate, found that the body weight, feed intake, FCR and daily BWGs were not significantly different among the experimental birds at 35 days of age, whereas body weight, daily BWGs and feed intake of the chickens receiving FWB with rumen filtrate at 5% inclusion rate were numerically higher than those in the other groups, followed by the chickens that received the 15% and 10% FWB with rumen filtrate,

respectively. Similarly, supplementation of enzymes to raw sorghum-based diets or addition of enzymes during reconstitution of sorghum did not further improve the growth performance of broilers (Manwar and Mandal, 2009). Leeson and Summers (2008) reported that WB has relatively high protein content and has a growth promoting effect. However, same authors reported that inclusion of WB alone and at low level will have positive effects on broilers and this effect is not connected the fiber content of WB. Darwazeh (2010) suggested that inclusion of FWB with rumen filtrate up to 15% of broiler finisher diet did not have any adverse effect in the performance of broilers. Tyagi *et al.* (1997) reported that the chicks fed on water-soaked wheat exhibited slightly higher weight gain than the chicks fed on untreated wheat based diet. It appears that the soaking of WB probably increased the availability of starch to enzymatic degradation leading to improved performance of the chicks through better energy utilization. It is inferred that the water soaking and oven drying of FWB improved the utilization of WB by growing chicks. Choct and Annison (1991) observed that the addition of wheat-isolated pentosans to a diet of broiler chicks caused a significant reduction in nutrient digestibility (particularly starch), weight gain and ME utilization by the chicken. Feed conversion ratios were also significantly poor by raw wheat diets, compared to the control diet. Feed intake showed a decreasing trend as the level of unprocessed wheat increased in diets. However, in present study, in overall trend, feed intake was comparable at different inclusion levels of wheat bran either raw or fermented in broiler diets (Fig. 4.3.2).

Present observations on FCR corroborated with the results of Manwar and Mandal (2009) who found that during 0-3 week of age, the FCR of the birds fed diet containing raw or reconstituted wheat with or without enzymes was statistically similar ($P>0.05$) to those

on control diet. In present study also, the FCR was statistically similar (P>0.05) during 3-6 weeks of age in chicks fed wheat bran with or without enzyme supplementation. However, addition of enzymes or reconstitution with or without enzymes significantly improved the FCR over the raw wheat based diets in similar age group of chicks (Manwar and Mandal, 2009). The data suggested that as the age of the chicks advanced, there was hardly any significant effect of enzyme supplementation on FCR. On the contrary, the earlier reports in the literature (Manwar and Mandal, 2009) showed that the enzyme supplementation did not prove beneficial to improve FCR. Conflicting reports are available in the literature regarding the effect of enzyme supplementation to wheat-based diets. McCracken and Quintin (2000) reported that enzyme addition improved live weight gain and gain:feed but no significant effect of wheat or enzyme addition on AME contents of the diet. Preston *et al.* (2001) did not observe much improvement in the growth rates of broilers fed wheat diets supplemented with feed enzyme (*Avizyme* -1300). During 0-6 week of age, better FCR was emanated from groups fed on fermented wheat bran, which was numerically better than control and raw group (Fig. 4.3.3). The various treatments including water treatment, enzyme supplementation and antibiotic addition were proved beneficial for improving the nutritive value of wheat (Adams and Naber, 1969; Misir and Marquardt, 1978; Friesen *et al.*, 1992). The higher body weights and gain in weight of broilers fed reconstituted wheat containing diets can be attributed to significantly higher feed conversion ratio and reduced intestinal viscosity. Bedford (1996) reported high positive correlation between FCR and intestinal viscosity since increasing viscosity could depress FCR as well as poultry production. Decreasing growth rate and FCR could be attributed to high intestinal viscosity (Wiseman, 2000).

FWB inclusion level at 5 and 7.5% numerically improved the growth performance of chicks over the control and raw groups in present study. These results are in line with Dinani *et al.* (2010), who observed that 7.5 and 15% inclusion level of toasted guar meal (TGM) with fermentation (D_7 and D_8 groups) showed better performance in quails over raw TGM. Moreover, Nagra (1984) reported 24% level of *Aspergillus niger* fermented guar meal showed better performance as compared to toasted and autoclaved guar meal. Verma and Singh (1985) reported that 13% CP of the diet from fermented guar meal showed positive growth in broiler.

Protein and energy efficiency

The data on effect of fermentation of WB with or without enzymes on protein and energy efficiency have been presented in Table 4.3.5 and the analyses of variance of the same in Table 4.3.5a.

On cumulative basis, it is evident that there were no significant differences in protein and energy efficiency of broiler chicks under different inclusion levels of either raw WB or FWB, with or without enzymes through feed during 0-3 (starting phase) and 3-6 (finishing phase) weeks of age. Similarly, during the 0-6 (overall phase) weeks of age, the cumulative protein and energy efficiency were also remained unaffected due to dietary treatments. In contrast to present results, Manwar and Mandal (2009), though worked on reconstituted sorghum, opined that protein and energy efficiency in control and reconstituted sorghum groups was better ($P<0.05$) as compared to unprocessed sorghum fed groups, during the starter phase. However, such trend could not be observed during finisher phase. The cumulative protein and energy efficiency was unaffected due to dietary treatments during the 0-6 week's period. Protein utilization efficiency

was better in birds fed reconstituted sorghum in comparison to those fed untreated sorghum. (Manwar and Mandal, 2009). Choct and Anniston (1990) reported that wheat pentosans decreased the protein utilization by increasing the endogenous amino acid secretions and as well as by inhibiting the true digestibility. These NSPs are poorly digested by poultry (about 12%), resulting into reduced energy utilization and interfere with the digestibility of other intrinsic feed components. Inclusion of high levels of wheat gave rise to higher *in vivo* viscosity (Allen *et al.*, 1997). Added pentosans from wheat based diets reduced digestibility by 20% (Fengler *et al.*, 1988).

In the present study, the protein and energy efficiency was statistically non-remarkable and was not affected by either fermentation or levels of inclusion of wheat bran with or without enzyme supplementation. However, Manwar and Mandal (2009) concluded that among birds fed raw wheat, overall protein and energy efficiency became poorer with the increased level of wheat in diets. Digestibility studies with wheat usually revealed a starch digestibility between 0.93 and 0.98 (Choct *et al.*, 1999; Annison, 1990). Svihus and Heltland (2001) indicated that an overload of wheat starch in the digestive tract might be the cause of poor digestibility for broilers. Wyatt *et al.* (1999) reported that the diets supplemented with enzymes increased the energy availability by 3.3%. It has been reported that the enzymes improved the utilization of nutrients and improved feed efficiency by degrading the viscous polysaccharides present in wheat (Choct and Annison, 1991). Thus, the results of the present study indicate that the birds fed FWB @ 5 and 7.5% inclusion level might be good for protein and energy efficiency as compared to the respective raw counterparts.

Table 4.3.5: Effect of feeding fermented wheat bran on protein efficiency (CP intake: gain, g) and energy efficiency (kcal ME intake: gain, g) of broilers at different growth phases

Diet	Treatment	Protein efficiency			Energy efficiency		
		0-3 wk	3-6 wk	0-6 wk	0-3 wk	3-6 wk	0-6 wk
D_1	WB_0	0.33	0.36	0.35	4.40	5.65	5.23
D_2	WB_5	0.33	0.36	0.35	4.39	5.68	5.25
D_3	$WB_{7.5}$	0.34	0.37	0.36	4.44	5.78	5.33
D_4	WB_0+E	0.32	0.37	0.35	4.26	5.79	5.26
D_5	WB_5+E	0.34	0.36	0.35	4.47	5.64	5.24
D_6	$WB_{7.5}$+E	0.32	0.36	0.35	4.28	5.63	5.16
D_7	FWB_5	0.32	0.35	0.34	4.17	5.53	5.03
D_8	$FWB_{7.5}$	0.33	0.35	0.35	4.35	5.51	5.14
SEM		0.0026	0.0025	0.0020	0.0319	0.0353	0.0280
Stat. significance		NS	NS	NS	NS	NS	NS

WB - Wheat bran, FWB – Fermented Wheat bran
NS- Non-significant (P>0.05)

4.3.2 Feed cost of broiler production

The data on feed cost of broiler production have been presented in Table 4.3.6. and the analyses of variance of the same in Table 4.3.6a.

The cost of feed was reduced (P<0.05) on fermentation of wheat bran over corresponding raw groups. However, the cost was comparable with the control group. On basis of weight gain and per kg live body weight the feed cost decreased in FWB over respective raw WB groups. The FWB inclusion at 5% level significantly reduced (P<0.05) the cost of feed per kg meat yield as compared to all other dietary treatments. Similarly, Manwar and Mandal (2009) found that the cost of feed was reduced as the level of sorghum increased in the diet replacing maize as sorghum is cheaper than maize. The feed cost calculated on the basis of weight gain or meat yield

remained similar (P>0.05) for all the diets. They further stated that differences in the price of maize and sorghum compensated the increase in the weight gain. However, in present study, the feed price reduced significantly (P<0.05) in FWB over the corresponding raw groups with or without enzyme supplementation.

The addition of enzymes was also not beneficial in terms of economics of broiler production in raw wheat bran based diets. However, the feed cost per kg body weight gain or meat yield was lower in FWB fed groups. Similar trend was observed by Manwar and Mandal (2009). It is concluded that, the feed cost per unit weight gain or meat yield apparently reduced due to fermentation.

Table 4.3.6: Effect of feeding fermented wheat bran on feed cost (Rs.) of broiler production

Diet	Treatment	Feed cost /kg gain			Feed Cost	Cost/kg
		0-3 wk	3-6 wk	0-6 wk	/kg BWt	meat
D_1	WB_0	25.32^{abc}	31.94	29.70^{cd}	28.85^{cd}	36.95^{ab}
D_2	WB_5	26.03^{abc}	33.61	31.11^{abc}	30.30^{abc}	38.87^{a}
D_3	$WB_{7.5}$	26.97^{a}	34.40	31.91^{a}	30.96^{a}	38.35^{a}
D_4	WB_0+E	24.76^{bc}	32.97	30.15^{bcd}	29.09^{bcd}	36.99^{ab}
D_5	WB_5+E	26.72^{a}	33.66	31.27^{ab}	30.66^{a}	38.79^{a}
D_6	$WB_{7.5}$+E	26.21^{ab}	33.77	31.13^{abc}	30.34^{ab}	37.69^{ab}
D_7	FWB_5	24.42^{c}	32.49	29.56^{d}	28.80^{d}	36.22^{b}
D_8	$FWB_{7.5}$	25.96^{abc}	32.61	30.47^{abcd}	29.98^{abcd}	37.82^{ab}
SEM		0.219	0.229	0.196	0.202	0.240
Stat. significance		P<0.05	NS	P<0.05	P<0.05	P<0.05

WB - Wheat bran, FWB – Fermented Wheat bran, BWt- Final body weight.

NS- Non-significant (P>0.05).

4.4.3 Nutrient utilization

The data on nitrogen, dry matter and energy utilization and retention of calcium and phosphorus have been presented in Table 4.2.7 and the analyses of variance of the same in Table 4.2.7a.

The N-retained (g/bird/d) and nitrogen retention (%) was significantly ($P<0.01$) improved due to fermentation (FWB at 5% inclusion level), as compared to raw counterparts with or without enzymes supplementation. However, no beneficial effect of enzyme supplementation was evident on the said parameter. The excreta DM percentage was statistically non-significant amongst different treatments. However, the DM metabolizabilty ($P<0.01$) and gross energy metabolizability differed significantly ($P<0.05$) due to the dietary treatments. The parameters responded positively to the treatments and were improved over the control; except in RWB with enzyme supplementation. When birds fed diets containing FWB has generally better ($P<0.05$) AMEn values than control group. Contrary to our results, nitrogen retention and DM metabolizabilty did not differ due to the dietary treatments (Manwar and Mandal, 2009). However, gross energy metabolizability, AMEn values and excreta dry matter differed ($P<0.05$) due to the dietary treatments. The improvement in AMEn values was significant ($P<0.05$) in diets containing sorghum reconstituted with enzymes.

When different sorghum types were compared, the gross energy metabolizability and the AMEn values improved ($P<0.01$) due to reconstitution with or without enzymes compared to all other types of grains. The per cent excreta dry matter in contrary to the present findings was significantly increased ($P<0.01$) due to enzyme supplementation to raw sorghum grains as well as due to addition of enzymes during the process of reconstitution over their respective counterparts (Manwar and Mandal, 2009). Viveros *et al.* (1994) have shown that supplementation of diet containing NSP with fiber-degrading enzymes improves MEn values of diets. Gross energy metabolizability and AMEn values were significantly higher in diets

Table 4.3.7: Effect of feeding fermented wheat bran on nutrient utilization by broilers

Diet	Treatment	N-retd. (g/b/d)	N-ret.(%) (%)	ExcretaDM (%)	DMM (%)	GEM (%)	AMEn	Difference (%)	Ca-ret. (%)	P-ret. (%)
D_1	WB_0	1.68^{bc}	54.09^{ab}	43.28	73.93^{bc}	73.50^{bc}	3068^{abc}	2.27^{bc}	30.61^{d}	41.23^{c}
D_2	WB_5	1.47^{cd}	52.34^{bc}	44.02	75.05^{ab}	74.43^{ab}	3025^{cd}	0.88^{c}	31.87^{bcd}	43.25^{b}
D_3	$WB_{7.5}$	1.70^{ab}	52.86^{ab}	43.84	74.86^{ab}	74.16^{abc}	2997^{d}	1.35^{c}	30.76^{d}	43.16^{b}
D_4	WB_0+E	1.42^{d}	46.38^{d}	44.54	72.94^{c}	73.14^{c}	3069^{abc}	2.27^{bc}	31.59^{cd}	42.98^{b}
D_5	WB_5+E	1.70^{ab}	53.69^{ab}	40.70	75.34^{a}	74.73^{a}	3110^{a}	3.71^{ab}	32.73^{abc}	44.25^{b}
D_6	$WB_{7.5}$+E	1.44^{d}	47.26^{cd}	38.01	73.03^{c}	73.30^{bc}	3098^{ab}	4.77^{a}	33.11^{ab}	43.86^{b}
D_7	FWB_5	1.92^{a}	58.17^{a}	40.24	74.45^{ab}	74.30^{ab}	3096^{ab}	3.11^{b}	34.11^{a}	46.97^{a}
D_8	$FWB_{7.5}$	1.78^{ab}	51.88^{bc}	43.28	74.01^{bc}	74.21^{abc}	3052^{bc}	2.26^{bc}	34.14^{a}	45.72^{a}
SEM		0.037	0.826	0.784	0.188	0.146	8.073	0.264	0.271	0.326
Significance		P<0.01	P<0.01	NS	P<0.01	P<0.05	P<0.01	P<0.01	P<0.01	P<0.01

WB - Wheat bran, FWB – Fermented Wheat bran. NS- Non-significant (P>0.05).

containing wheat reconstituted with enzymes compared to their untreated counterparts. McCracken and Quintin (2000) observed no significant effect of wheat or enzyme addition on AME contents of the diet. However, wheat processed or unprocessed when incorporated at 100% replacement level of maize tend to decrease the dry matter metabolizability, nitrogen retained per bird and AME values (both classical and nitrogen corrected). The NSPs in wheat exhibit anti-nutritive activity when present in poultry diet (Saki, 2005). The high levels of arabinoxylan (pentosans) in wheat were responsible for low metabolizable energy value (Choct and Annison, 1990). Water-extractable and alkali-extractable fractions when added to a commercial type broiler diet to provide 25.9-65.7 g arabinoxylan/kg, a significant dose-dependent depression in AME value of the diet occurred and at highest levels of inclusion the ileal digestibility of starch, protein and lipid were reduced from 96 to 82%, 75 to 61% and 93 to 69%, respectively (Mollah *et al.*, 1983).

The improvement in Ca and P-retention in present study were significant ($P<0.01$) in diets containing FWB over the raw counterparts and control. However, Dinani (2009) studied certain parameters in quails wherein, nutrient utilization indicated that 15% TGM without enzyme showed comparatively poor nutrient utilization in terms of DM, energy, AME metabolizability, N, Ca and P retention as compared to other dietary treatments. Further, enzyme supplemented groups showed better nutrient utilization as compared to unsupplemented groups and FTGM showed better nutrient utilization as compared to enzyme supplemented groups. Gunashree *et al.* (2007) found that *A. niger* produce higher amount of phytase enzyme in solid substrate fermentation, which is active in the hydrolysis of phytic acid component of plant products. Birds supplemented with enzyme showed

14% decrease in P excretion in fecal matter and 25% deposition of P content in thighs and leg bones. Similarly, Ca content of the same bone showed an increased trend. It is inferred that, diet containing FWB found beneficial effect for nutrient utilization and improved the Ca and P retention due to fermentation.

4.3.4 Carcass traits

The influence of feeding FWB and raw WB with or without enzyme to broilers chickens up to 6 weeks of age on various carcass traits (pre-slaughter fasting live weight, dressing percentage, blood loss, feather loss, eviscerated weight, giblet yield, ready-to-cook-yield and abdominal fat), yield of cut-of-parts (breast, drumsticks, thighs, back, neck and wings) and organs weights (gizzard, heart, liver, spleen and bursa) of broilers (as % live weight) at the end of feeding trial (42^{nd} day) were studied and the results are presented hereunder.

Slaughter traits

The data on effect of dietary treatments on blood and feather loss, eviscerated yield, edible yield, giblet yield, ready-to-cook and abdominal fat pad (% live weight) of broilers are presented in Table 4.3.8 and the analyses of variance of the same in Tables 4.3.8a (given as in Annexure). The analysis of variance for slaughter traits of broilers revealed a non significant influence of treatments on blood loss, giblet yield and abdominal fat pad. However, feather loss, eviscerated yield, edible yield, and ready-to-cook (eviscerated with giblet weight) yield was significantly ($P<0.01$) different amongst various treatments. There was a significant ($P<0.01$) improvement in the said parameters (eviscerated and edible yield) in fermented wheat bran as compared to the control and raw counterparts. Similarly, the parameters also improved in enzyme supplemented groups. Likewise, the ready-to-cook yield followed similar trend.

Table 4.3.8: Effect of feeding fermented wheat bran on carcass traits (% live weight) of broilers at 6 weeks of age

Diet	Treatment	Bloodloss	Featherloss	Eviscerated yield	Edibleyield	Gibletyield	Ready-to-cook	Abd.fat pad
D_1	WB_0	3.96	7.75^{a}	65.51^{c}	71.92^{c}	5.05	70.55^{b}	1.26
D_2	WB_5	3.92	6.63^{c}	65.24^{c}	71.96^{c}	5.30	70.54^{b}	1.25
D_3	$WB_{7.5}$	4.00	6.53^{c}	66.90^{abc}	73.63^{ab}	5.14	72.04^{a}	1.17
D_4	WB_0+E	4.52	5.64^{d}	66.00^{bc}	72.80^{bc}	5.37	71.37^{ab}	1.21
D_5	WB_5+E	4.74	6.74^{bc}	66.85^{abc}	73.30^{ab}	5.19	72.04^{a}	1.28
D_6	$WB_{7.5}$+E	4.78	7.82^{a}	67.52^{ab}	74.10^{ab}	5.18	72.70^{a}	1.23
D_7	FWB_5	4.49	7.49^{ab}	68.03^{a}	73.54^{ab}	4.71	72.74^{a}	1.30
D_8	$FWB_{7.5}$	4.36	7.14^{abc}	67.53^{ab}	74.50^{a}	5.19	72.72^{a}	1.58
SEM		0.090	0.126	0.221	0.186	0.067	0.191	0.034
Stat. significance		NS	P<0.01	P<0.01	P<0.01	NS	P<0.01	NS

WB - Wheat bran and FWB – Fermented Wheat bran
NS- Non-significant (P>0.05)

The findings are in accordance with Manwar and Mandal (2009) who found that inclusion of wheat and its processed forms in broiler diets could not exert any significant influence on per cent blood loss, giblet yield and abdominal fat pad. The results pertaining to carcass characteristics were similar to findings of Rama Rao *et al.* (2002), Tyagi *et al*. (2003) and Sannamani (2002) who reported that feeding of sorghum did not affect blood loss. Also, blood loss did not differ significantly ($P>0.05$) between treatments groups in broiler quail when fed with fermented TGM with or without enzymes supplementation. Dressing and eviscerated % was significantly ($P<0.05$) improved in FTGM and their corresponding counterparts with enzyme supplementations as compared to control (Dinani, 2009). However, per cent giblet yield was lower ($P<0.01$) in groups fed on diets containing 75 or 100% reconstituted wheat instead of maize in comparison to control group. In present study the feather loss and edible yield were relatively higher in FWB group. Contrary to this, feeding of sorghum did not affect feather loss and edible yield (Manwar and Mandal, 2009). Likewise, the eviscerated yield and ready-to-cook (eviscerated with giblet weight) were significantly more in FWB group in present study. Contrary to this, Manwar and Mandal (2009) observed that feeding of sorghum did not affect eviscerated and ready-to-cook yield. The wheat and its processed forms resulted in a non significant difference on per cent eviscerated yield, edible yield, and ready-to-cook (eviscerated with giblet weight) yield and other carcass traits (Manwar and Mandal, 2009). Bhutia (2006) reported that inclusion level of TGM up to 10% in broiler quail ration with or without enzyme did not significantly ($P<0.05$) effect the dressed and eviscerated weight.

Cut-up parts

The relative mean values of cut-up parts of carcass (% live weight) measured at the end of 6th weeks of age of broilers under different treatments are given in Table 4.3.9 and the analyses of variance of the same in Tables 4.3.9a.

The results revealed that the weights of breast, back and neck did not differ significantly ($P>0.05$) amongst treatments. The relative breast, back and neck weights influenced by different inclusion levels of raw WB, with or without enzymes and FWB were also found to be similar in all treatment groups. The relative drumsticks, thighs and wings weights influenced by different inclusion levels of raw WB, with or without enzymes and FWB were found significantly ($P<0.01$) different amongst treatments. The relative weight of drumstick was significantly ($P<0.01$) higher in FWB group than the control and their raw counter parts. Likewise, same trend was followed in weight of wings which was significantly higher in FWB and in raw WB with enzyme supplementation. Moreover, the mean weights of thighs were significantly differing but no consistent trend could be observed. The results of some indirect studies indicated that inclusion of wheat either raw or processed did not alter carcass traits in broilers (Manwar and Mandal, 2009). In this study breast weight remained statistically similar among the carcass cut-up parts, whereas the wings weighed statistically more in FWB group. Similar result was observed in reconstituted sorghum group at 100% level (Manwar and Mandal, 2009). However, the present results on breast yield are contrary to Manwar and Mandal (2009).

Table 4.3.9: Effect of feeding fermented wheat bran on cut-up parts (% eviscerated weight) in broilers at 6 weeks of age

Diet	Treatment	Breast	Drumstick	Thigh	Back	Neck	Wings
D_1	WB_0	25.09	13.80^{d}	15.37^{a}	24.52	7.00	12.98^{b}
D_2	WB_5	25.81	13.88^{cd}	15.22^{a}	23.72	6.80	12.91^{b}
D_3	$WB_{7.5}$	25.59	14.07^{cd}	14.78^{abc}	24.28	6.40	12.98^{b}
D_4	WB_0+E	24.85	14.53^{abc}	15.07^{ab}	24.11	6.51	13.85^{a}
D_5	WB_5+E	24.72	14.85^{ab}	13.93^{cd}	25.44	6.79	13.73^{a}
D_6	$WB_{7.5}$+E	23.82	14.23^{bcd}	14.64^{abcd}	24.96	6.64	13.31^{ab}
D_7	FWB_5	25.23	14.80^{ab}	13.83^{d}	24.61	6.32	13.71^{a}
D_8	$FWB_{7.5}$	24.10	15.07^{a}	14.31^{bcd}	25.42	6.90	13.56^{ab}
SEM		0.205	0.094	0.117	0.169	0.084	0.084
Stat. significance		NS	P<0.01	P<0.01	NS	NS	P<0.01

WB - Wheat bran and FWB - Fermented wheat bran
NS - Non-significant (P>0.05)

Organ weights

The results on the effect of feeding FWB *vis-a-vis* WB with or without enzyme supplementation to broiler chickens up to 6 weeks of age on relative weight of vital and immune organs (% live weight) are presented in Table 4.3.10. The analysis of variance of gizzard, heart and liver as per cent live weight showed a non significant difference due to different inclusion levels of raw WB, with or without enzymes and FWB (Table 4.3.10a). Likewise, the mean weights of Bursa of fabricus noted a non significant difference in all dietary treatments. However, the weight of spleen was significantly (P<0.05) different amongst various dietary treatments. The birds of fermented WB and WB with enzyme supplemented recorded significantly (P<0.05) higher weights of spleen than control and their raw counterparts.

The results pertaining to organ weights are similar to the earlier reports (Rama Rao *et al.*, 2002; Sannamani, 2002, Tyagi *et*

al., 2003; Manwar and Mandal, 2009). Feeding of FWB at different inclusion level did not affect heart, liver and gizzard weight except spleen weights, which was reduced in groups, fed on diets containing raw WB. In accordance to Manwar and Mandal (2009) reported that the mean weight of heart, liver and gizzard did not significantly differ due to inclusion of raw sorghum in broiler diet. However, the lower relative spleen weight was recorded in group fed raw wheat at 50% inclusion level of maize but no definite trend was observed. Moreover, Sannamani (2002) reported significantly higher weight of gizzard in broilers fed red sorghum based diet which was attributed to lower dietary energy level on inclusion of sorghum in diet as the red sorghum contains much less than in its white variety (Mandal *et al.*, 2006). Nelson *et al.* (1975) also reported the higher weight of gizzard and it might be due to higher amount of crude fiber. However, this change was not appeared in FWB fed groups at both 5 and 7.5% inclusion level (Table 4.3.10). It was reported that most of the digestive organ weights were higher in broilers fed with sorghum based diets (Dixit and Baghel, 1998). It was also reported that the liver, gizzard, giblet and spleen weights were affected significantly by feeding of sorghum in broiler chicks (Attia and Rahman, 1996).

Liver weight was significantly ($P<0.05$) higher in 15% TGM without and with enzyme (D_3 and D_6) groups as compared to control and other groups. Our results are in line with Lee *et al.* (2003a) and Bhutia (2006). Lee *et al.* (2003a) found that relative weights of liver was significantly ($P<0.05$) increased by the inclusion of hull fraction of guar meal at 7.5% and 10% levels. Bhutia (2006) reported inclusion of 10% TGM with or without enzyme supplementation in

the diets increased liver weight as compared to diet containing 0, 5 and 7.5% TGM in growing quails. However, in contrary, Bakshi *et al.* (1964), Prakash and Singh (1984) and Nagra (1984) reported that liver weight were found to be unaffected by the feeding of guar meal to chicks.

The present results are in accordance with the finding of Vohra and Kratzer (1964a,b) and Prakash and Singh (1984) who reported that the guar meal feeding to chicks resulted in increase in pancreas weight. Couch *et al.* (1967) also reported the hypertrophy of pancreas resulted in the chicks fed 20 and 30% processed guar meal and in all groups fed raw guar meal. Lee *et al.* (2003a) also found that the relative weights of pancreas were significantly higher by the inclusion of hull fraction of guar meal at 10 and 7.5% inclusion. Similarly, Bhutia (2006) reported that pancreas weight was significantly higher in diet containing 10% TGM with or without enzyme in broiler quail ration.

The relative weight of gizzard, heart, spleen and bursa of Fabricius did not show any specific trend to various dietary treatments. Gizzard, heart, spleen and bursa of Fabricius weight did not differ significantly ($P>0.05$) between groups. The result of present study are in agreement with findings of Bakshi *et al.* (1964) and Brahma *et al*. (1979a,b) who reported that visceral organ weights were comparable by the inclusion of TGM in the diets of broiler chicken. Similarly, Bhutia (2006) reported gizzard, heart, spleen weight did not differ significantly ($P<0.05$) at 0, 5 and 7.5% TGM levels with or without enzyme supplementation in broiler quail ration.

Table 4.3.10: Effect of feeding fermented wheat bran on vital organs and immune organs (% live weight) of broilers at 6 weeks of age

Diet	Treatment	Gizard	Heart	Liver	Spleen	Bursa
D_1	WB_0	2.39	0.52	2.14	0.23^{abc}	0.25
D_2	WB_5	2.51	0.53	2.27	0.24^{abc}	0.29
D_3	$WB_{7.5}$	2.45	0.56	2.12	0.19^{c}	0.25
D_4	WB_0+E	2.42	0.52	2.43	0.25^{ab}	0.26
D_5	WB_5+E	2.56	0.51	2.12	0.21^{bc}	0.21
D_6	$WB_{7.5}$+E	2.57	0.53	2.08	0.19^{bc}	0.34
D_7	FWB_5	2.28	0.48	1.94	0.21^{bc}	0.23
D_8	$FWB_{7.5}$	2.49	0.48	2.22	0.27^{a}	0.29
SEM		0.042	0.008	0.040	0.006	0.015
Stat. significance		NS	NS	NS	P<0.05	NS

WB - Wheat bran, FWB – Fermented Wheat bran
NS- Non-significant (P>0.05).

4.3.5 Serum biochemical and immune parameters

The data pertaining to effect of dietary treatments on serum total cholesterol, triglycerides and uric acid, cell mediated immune response to PHA-P and humoral immune response to SRBC are presented in Table 4.3.11 and the analyses of variance of the same in Tables 4.3.11a.

The level of serum cholesterol, triglycerides and uric acid was found to remain unaffected by the different inclusion levels of raw WB, with or without enzymes and FWB. When considering, immune parameters, the HA titer of fermented WB group remained statistically similar amongst different treatments. However, the CMI response of birds revealed significant (P<0.01) differences amongst all dietary treatment. The serum triglyceride value of D_7 (FWB5) group was numerically higher than D_1 (control) group and the rest of the groups. Nevertheless, compared to the control group, cholesterol and uric acid values were comparable and no definite

trend was evident in other dietary treatment groups. It was observed that the CMI response of broiler chicken was significantly higher ($P<0.01$) in D_7 (FWB_5) group than the control and raw WB with or without enzyme supplemented group. When the types of WB compared, the birds fed diets containing FWB or untreated WB with or without enzymes showed improved ($P<0.01$) immune response measured in terms of cell mediated immune response to PHA-P compared to control group. Compared to the control group, there were no statistical significant differences in terms of humoral response to SRBC in all the groups.

Table 4.3.11: Effect of fermented wheat bran on certain serum (mg/100ml), immune parameters and caeca microbial count (cfu/g) of broilers

Diet	Treatment	Cholesterol	Triglycerides	Uric acid	CMI response (mm)	HA titer (log 2)	Caeca microbes TPC (cfu/g)
D_1	WB_0	102.63	77.35	6.66	0.32^{c}	5.82	63.33^{a}
D_2	WB_5	112.50	78.07	7.51	0.36^{b}	7.45	61.83^{a}
D_3	$WB_{7.5}$	124.20	78.37	7.91	0.38^{ab}	7.20	57.17^{a}
D_4	WB_0+E	124.20	79.39	7.85	0.37^{ab}	7.07	53.50^{ab}
D_5	WB_5+E	108.06	74.39	7.32	0.36^{ab}	6.45	43.67^{abc}
D_6	$WB_{7.5}$+E	124.06	72.14	8.76	0.37^{ab}	7.70	35.33^{bc}
D_7	FWB_5	113.49	81.34	7.14	0.39^{a}	7.82	31.17^{c}
D_8	$FWB_{7.5}$	117.51	80.72	7.32	0.38^{ab}	7.70	33.67^{c}
SEM		2.724	1.095	0.216	0.004	0.187	3.122
Stat. significance		NS	NS	NS	P<0.01	NS	P<0.01

WB - Wheat bran, FWB – Fermented wheat bran and TPC- Total plate count.
NS- Non-significant (P>0.05).

There are hardly any studies in the literature involving effect of feeding of fermented WB with *A. niger* to broiler chicken, in regards to serum biochemical and immune response have been carried out earlier. Present results are similar to the earlier

reports, Manwar and Mandal (2009), who worked on reconstituted sorghum, and opined that feeding processed sorghum did not influence the serum cholesterol, triglycerides and uric acid concentration. Similarly, humoral response to SRBC did not differ due to inclusion of reconstituted sorghum and their raw counterparts in broiler diets. However, the present results on CMI response of birds are contrary to Manwar and Mandal (2009) wherein PHA-P was better in sorghum-fed groups. Moreover, same authors suggested further that when the types of wheat compared, the birds fed diets containing wheat reconstituted with or without enzymes showed increased ($P<0.01$) immune response measured in terms of humoral response to SRBC compared to their counterparts fed raw or supplemented with enzymes.

Due to fermentation, the serum biochemical parameters such as total cholesterol, triglycerides and uric acid were not affected due to dietary treatments. The cell-mediated immune response to phytohaemagglutinin-P was better in all WB-fed groups. However, the humoral response to sheep red blood cells remained unaffected owing to the raw WB or its fermented form. Thus it signifies the superior cellular immune response in broiler chickens can be achieved by feeding of fermented WB.

4.3.6 Caeca microbial count

The mean total microbial counts of caeca contents (cfu/g) of broiler as influenced by dietary levels of WB with or without enzyme supplementation and FWB have been presented in Table 4.3.11; along with their analysis of variance in annexure Table 4.3.11a.

Caeca microbial count ranged from 63.33 in control diet (D_1) to 31.17 in 5% FWB (D_7) group. Caeca microbial count was

significantly ($P<0.01$) higher in control (D_1) as compared to other groups. Enzyme supplemented groups along with WB (D_4, D_5, D_6) significantly ($P<0.01$) reduced caeca microbial count as compared to their respective non-enzyme supplemented groups (D_1, D_2 and D_3). FWB at 5 and 7.5% (D_7 and D_8) reduced caeca microbes significantly ($P<0.01$) as compare to control and other dietary groups. The present study are in line with Bailey *et al*. (1991), Ishihara *et al.* (2000), Lee *et al.* (2003b), Gujral (2005), Bhutia (2006) and Dinani (2009). Bailey *et al*. (1991) reported that chicken fed fructo-oligo-saccarides (FOS) had four fold reduction in the level of Salmonella present in the caeca. Ishihara *et al*. (2000) who reported that partially hydrolyzed guar gum prevented colonization of *Salmonella enteriditis* in young and laying hen. Gujral (2005) reported that the fructo-oligo-saccharides (FOS) and mannan oligo-saccharides (MOS) reduced significantly ($P<0.05$) with caecal colonization of *Salmonella typhimurium* upon challenge with live bacteria on third day of age in broiler quails. Eeckhaut *et al.* (2008) reported that arabinoxylooligosaccharides released from WB with or without enzyme supplementation in broiler chicken significantly ($P<0.05$) reduced caeca microbial count of Salmonella as compared to control. They further deduced that arabinoxylooligosaccharides in the substrate WB behaved as prebiotics and enhanced the population of beneficial bacteria like Bifidobacteria that consequently would have lead to reduction in caeca count of Salmonella in broilers.

4.3.7 Sensory evaluation

The data on various sensory characteristics i.e. appearance, flavour, juiciness, tenderness and overall acceptability of broiler

meat as influenced by dietary inclusion of WB with or without enzyme supplementation and FWB are shown in Table 4.2.12 and the analyses of variance of the same in Table 4.2.12a. The appearance, flavour, juiciness, tenderness, texture and overall acceptability score of fresh cooked meat as judged by the panelists was not found to differ (P>0.05) by the various treatments of WB. The appearance score was comparable amongst various treatments of WB (P>0.05).

Table 4.3.12: Effect of feeding fermented wheat bran on sensory attributes of meat

Diet	Treatment	Appearance	Texture	Juiciness	Flavour	Tenderness	Accept.
D_1	WB_0	6.83	5.83	6.17	5.67	5.00	6.33
D_2	WB_5	6.50	6.83	6.50	5.33	6.83	6.67
D_3	$WB_{7.5}$	7.17	6.33	5.33	6.00	6.33	6.17
D_4	WB_0+E	5.83	6.50	5.50	5.67	7.00	6.67
D_5	WB_5+E	5.67	6.33	6.33	6.33	6.33	6.33
D_6	$WB_{7.5}$+E	6.67	6.33	7.33	6.33	6.00	6.67
D_7	FWB_5	6.33	6.00	7.33	6.00	6.33	6.67
D_8	$FWB_{7.5}$	6.67	6.00	6.67	6.00	7.17	7.00
SEM		0.255	0.181	0.221	0.146	0.191	0.174
Stat. significance		NS	NS	NS	NS	NS	NS

WB - Wheat bran and FWB – Fermented Wheat bran
NS- Non-significant (P>0.05)

It showed that inclusion of raw WB, with or without feed enzyme and FWB in broiler diets did not bring out much change in order to change the sensory quality attributes of meat. It also indicates that wheat bran NSPs may not be always alter the sensory quality of the meat. The present results are in agreement with Manwar and Mandal (2009a,b). However, it was reported that the sorghum-based diets did alter the pigmentation of skin in broilers (Sharda and Thakur, 1977).

4.4 EXPERIMENT 4: EFFECT OF FEEDING FERMENTED DORB WITH OR WITHOUT ENZYME ON GROWTH AND IMMUNE RESPONSE IN BROILERS

The present study was intended to enhance the availability of nutrients from de-oiled rice bran (50:50, w/v) through solid substrate fermentation with or without feed enzymes, and to examine the influence of fermentation of DORB with or without feed enzymes on the growth performance, gross energy, dry matter metabolizability, nitrogen retention, immune responsiveness and economics (feed-cost) of commercial (*CARI*-coloured) broiler chickens.

4.4.1 Growth performance

The result of the present study in the context of growth performance and related traits, influenced by the fermented de-oiled rice bran, with or without enzymes in diet of broiler chickens are presented and discussed below.

The growth performance of experimental broiler chicks was evaluated in terms of the gain in body weight, feed intake, and feed conversion ratio at weekly interval (0-6 weeks of age), and phase wise for instance starting phase (0-3 weeks of age), finishing phase (3-6 weeks of age) and overall phase (0-6 weeks of age).

Body weight gain, feed intake and feed conversion ratio

The data on mean body weight gain, feed intake and feed conversion ratio in different phases of growth of broilers as influenced by the dietary treatments have been depicted in Table 4.4.1, Tables 4.4.2 and 4.4.3 at weekly interval (0-6 weeks of age) and phase wise for instance starting phase (0-3 weeks of age), finishing phase (3-6 weeks of age) and overall phase (0-6 weeks of age), respectively and the analyses of variance of the same in Tables 4.4.1a, 4.4.2a and 4.4.3a, respectively.

On weekly basis, it is clear that there were no significant differences in body weight gains (BWGs) of broiler chicks under different treatments of DORB at included levels either 5% or 7.5% raw DORB and/or FDORB during 1st, 3rd, 4th and 5th week of age. However, during 2nd and 6th week of age, the mean BWGs were significantly different amongst treatments. During 2nd week, the birds fed FDORB at 5% inclusion level (D_7) had revealed a significantly ($P<0.01$) higher BWGs in comparison with the birds of control group (D_1), the birds fed raw DORB at 7.5% inclusion level (D_3) and that of 5% inclusion level with enzymes (D_5). However, during 6th week of age, the BWGs were significantly ($P<0.05$) higher in D_7, D_4, D_5, D_8, D_1 and D_2 as compared to D_3 and D_6. On cumulative analysis, BWGs of broilers were not affected by the both inclusion of raw, either with or without enzyme and fermented DORB during starting phase (0-3 weeks of age) and finishing phase (3-6 weeks of age). Similarly, during 0-6 (overall phase) weeks of age, mean BWGs amongst different treatments were statistically non-significant (Fig. 4.4.1). At 6th weeks of age, the mean BWG of the birds fed diets containing FDORB at 5% inclusion level (1418g) was numerically higher than control and other treatment groups. However, the body weight gain reduced with the increased level of raw DORB in diets. Fermentation of DORB improved weight gain in broilers as compared to untreated DORB, and was comparable to control diet containing corn-soya. The enzyme supplementation to the DORB based diets was not remarkable in terms of body weight gain in broilers.

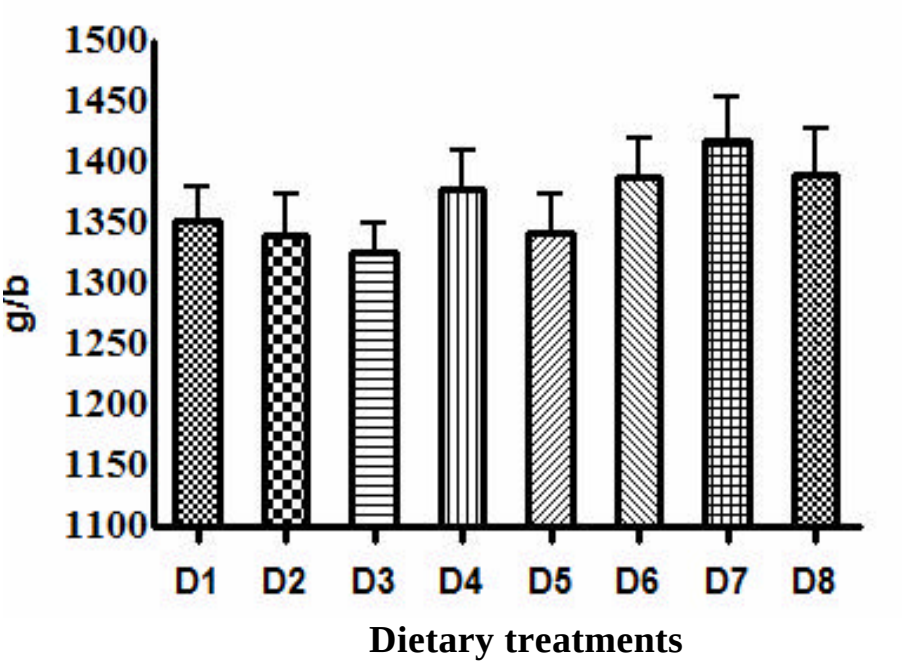
Table 4.4.1: Effect of feeding fermented de-oiled rice bran on body weight gain (g/b) of broilers at weekly intervals and different growth phases (wk)

Diet	Treatment	Body weight gain(g)								
		Weekly						Phase wise		
		1 wk	2 wk	3 wk	4 wk	5 wk	6 wk	0-3 wk	3-6 wk	0-6 wk
D_1	$DORB_0$	91	172^{d}	254	325	263	247^{ab}	517	835	1352
D_2	$DORB_5$	86	175^{bcd}	242	317	281	238^{ab}	503	836	1340
D_3	$DORB_{7.5}$	85	170^{d}	263	321	276	212^{b}	517	809	1327
D_4	$DORB_0$+E	94	187^{abc}	245	308	274	270^{a}	526	852	1379
D_5	$DORB_5$+E	87	169^{d}	256	311	280	240^{ab}	512	830	1342
D_6	$DORB_{7.5}$+E	94	188^{abc}	276	321	300	209^{b}	558	830	1388
D_7	$FDORB_5$	87	194^{a}	255	354	270	259^{a}	535	882	1418
D_8	$FDORB_{7.5}$	95	191^{ab}	266	308	303	226^{ab}	552	837	1389
SEM		1.09	2.05	3.20	3.93	3.95	5.08	5.32	8.57	11.28
Stat. significance		NS	P<0.01	NS	NS	NS	P<0.05	NS	NS	NS

DORB - De-oiled rice bran and FDORB – Fermented de-oiled rice bran
NS- Non-significant (P>0.05).

Data on mean feed intake of broilers obtained at weekly and cumulative intervals are presented in Table 4.4.2. Statistical analysis of the data pertaining to feed intake as influenced by different inclusion levels of either raw DORB or FDORB, with or without enzymes to broilers revealed that average weekly feed intake remained statistically non-significant for 1st and 4th weeks of age. However, during 2nd week of feeding trail, mean feed intake were significantly (P<0.01) lower at 235 g in D_7 than the rest of the groups except in D_2 ($DORB_5$) and D_8 ($FDORB_{7.5}$) group. The feed intake remained statistically similar in D_1, D_2, D_3, D_4, D_5 and D_6 groups. During, 3rd week of feeding trail, mean feed intake were significantly (P<0.05) lower at 375g in D7 than the rest of the groups except in D_1 ($DORB_0$), D_2 ($DORB_5$) and D_4 ($DORB_0$ + E) group. The feed intake remained statistically similar in D_3, D_5, D_6 and D_8 groups. Also,

Fig. 4.4.1 : Effect of feeding fermented de-oiled rice bran on body weight gain (g/b) in broilers (0-6 wk)



mean feed intake were significantly ($P<0.01$) lower at 522g in D_7 than the rest of the groups except in D_8 ($FDORB_{7.5}$) group. The feed intake remained statistically similar in D_1, D_2, D_3, D_4, D_5 and D_6 groups during 5th week of feeding trail. On the other hand, during 6th week of age, the feed intake were significantly ($P<0.01$) lower at 551g in D_8 ($FDORB_{7.5}$) except in D_7 ($FDORB_5$) group. The feed intake remained statistically similar in D_1, D_2, D_3, D_4, D_5 and D_6 groups. On cumulative basis, during 0-3 weeks of age, the feed intake was not found to be significantly affected by inclusion of raw and/or FDORB, with or without enzyme supplementation. However, though the feed intake did not differ significantly ($P>0.05$) during the first 3 weeks of age but was compensated in all the groups during finisher phase (3-6 weeks of age), wherein, the birds fed diets containing FDORB at 5% inclusion level (1676g) had statistically lower feed intake to that of containing untreated DORB, with or without enzyme supplementation. The feed intake of birds fed diets containing FDORB at 7.5% inclusion level was almost comparable. Rest of the groups recorded similar feed intake than those of control diet fed group. When different types of DORB were compared, the lower ($P<0.01$) feed intake was recorded due to fermentation. Similarly, during 0-6 weeks of overall phase, the mean feed intake was significantly different amongst treatments of broilers (Fig. 4.4.2). The mean feed intake was significantly ($P<0.01$) lower in D_7 ($FDORB_5$) than other dietary treatments except D_8 ($FDORB_{7.5}$). When birds fed diets containing untreated and enzyme supplemented DORB, the feed intake was comparable with control group. In general, overall feed intake was lower ($P<0.01$) in groups fed FDORB in comparison to their counterparts fed unprocessed DORB with or without enzyme supplementation.

Table 4.4.2: Effect of feeding fermented de-oiled rice bran on feed intake (g/b) of broilers at weekly intervals and different growth phases (wk)

Diet	Treatment	Feed intake (g)								
		Weekly						Phase wise		
		1 wk	2 wk	3 wk	4 wk	5 wk	6 wk	0-3 wk	3-6 wk	0-6 wk
D_1	$DORB_0$	132	257^{ab}	396^{ab}	648	600^{ab}	614^{ab}	785	1862^{ab}	2646^{ab}
D_2	$DORB_5$	121	250^{abc}	420^{ab}	665	655^{a}	664^{a}	791	1983^{a}	2773^{a}
D_3	$DORB_{7.5}$	122	265^{a}	429^{a}	640	636^{a}	668^{a}	816	1944^{a}	2760^{a}
D_4	$DORB_0$+E	136	265^{a}	419^{ab}	624	633^{a}	638^{a}	819	1895^{a}	2713^{ab}
D_5	$DORB_5$+E	139	263^{a}	441^{a}	646	613^{ab}	653^{a}	842	1912^{a}	2754^{a}
D_6	$DORB_{7.5}$+E	137	263^{a}	447^{a}	659	606^{ab}	670^{a}	847	1935^{a}	2780^{a}
D_7	$FDORB_5$	132	235^{c}	375^{b}	586	522^{c}	568^{bc}	741	1676^{c}	2418^{c}
D_8	$FDORB_{7.5}$	125	242^{bc}	432^{a}	642	549^{bc}	551^{c}	800	1741^{bc}	2541^{bc}
SEM		2.62	6.19	9.86	10.04	9.49	23.17	29.69	2.62	6.19
Stat. Significance		NS	P<0.01	P<0.05	NS	P<0.01	P<0.01	NS	P<0.01	P<0.01

DORB - De-oiled rice bran and FDORB – Fermented de-oiled rice bran
Mean values bearing different superscripts within a column differ significantly. NS- Non-significant (P>0.05).

The data on feed conversion ratio (FCR) calculated as feed consumption per unit weight gain at weekly and cumulative intervals of birds as influenced by different inclusion levels of either raw DORB or FDORB, with or without enzymes through feed has been summarized in Table 4.3.3. Statistical analysis of the mean FCR values of birds under different treatments revealed significant differences up to 6th weeks of age except during 1st week of age. The FCR values, however, during 1st week remained statistically similar amongst different treatment but was compensated in all the groups during finisher phase. On cumulative analysis, the mean FCR values of broilers were significantly different amongst various treatments during starting phase (0-3 weeks of age) and finishing phase (3-6 weeks of age). The chicks of D_7 group, in which the birds fed diets containing FDORB at 5% inclusion level, recorded significantly

Fig. 4.4.2 : Effect of feeding fermented de-oiled rice bran on feed intake (g/b) in broilers (0-6 wk)

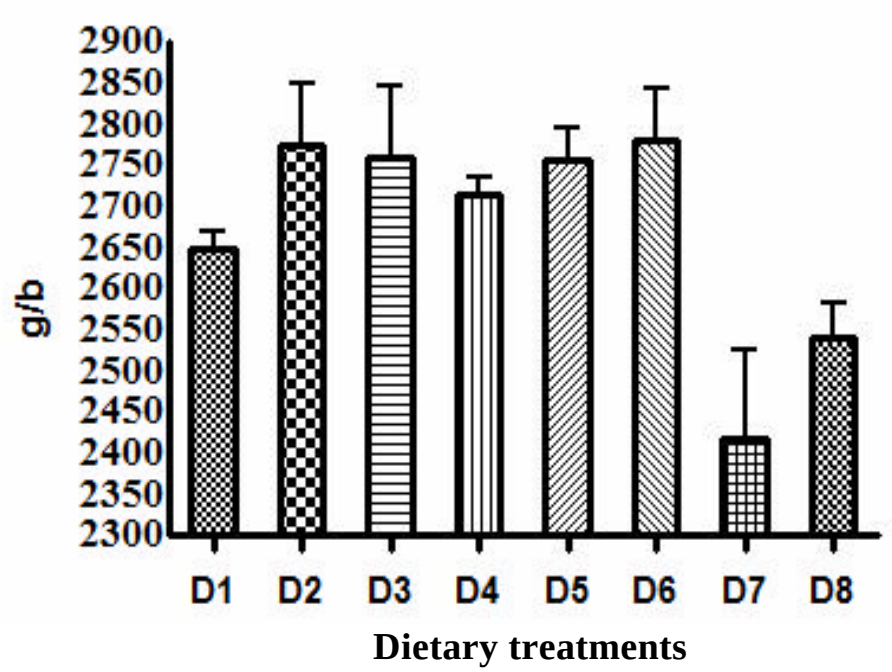
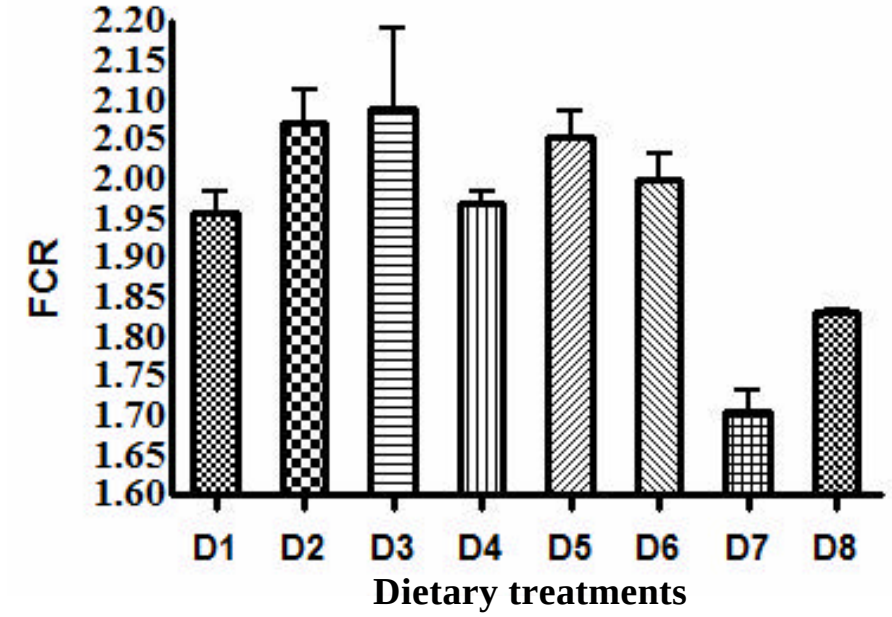


Fig. 4.4.3 : Effect of feeding fermented de-oiled rice bran on FCR (0-6 wk) of broilers



(P<0.05) better FCR value (1.38) and FCR value (1.91) than the control and their counterparts during starting and finishing phase, respectively. When birds fed diets containing untreated and enzyme supplemented DORB, the FCR values were comparable with control group. Similarly, during 0-6 (overall phase) weeks of age, mean FCR values amongst different treatments were statistically significant. Almost similar trend was maintained in FCR values during 0-6 weeks of age, wherein, the best FCR was emanated from groups fed on FDORB at 5% inclusion level (1.71), which differ significantly from control group (1.96) and their raw counterparts (Fig. 4.4.3). However, FCR value of D_7 group was comparable to the control group during 3-6 and 0-6 weeks of age. Due to fermentation of DORB, improvement in FCR in broilers was evident as compared to untreated DORB, which was comparable to control diet containing corn-soya diet.

Table 4.4.3: Effect of feeding fermented de-oiled rice bran on feed conversion ratio (FCR) of broilers at weekly intervals and different growth phases (wk)

Diet	Treatment	Feed conversion ratio (FCR)								
		Weekly						Phase wise		
		1 wk	2 wk	3 wk	4 wk	5 wk	6 wk	0-3 wk	3-6 wk	0-6 wk
D_1	$DORB_0$	1.45	1.50^{a}	1.56^{ab}	2.00^{a}	2.30^{a}	2.51^{b}	1.52^{abc}	2.23^{ab}	1.96^{ab}
D_2	$DORB_5$	1.41	1.43^{ab}	1.73^{a}	2.11^{a}	2.34^{a}	2.80^{ab}	1.57^{ab}	2.37^{a}	2.07^{a}
D_3	$DORB_{7.5}$	1.44	1.58^{a}	1.64^{a}	2.01^{a}	2.31^{a}	3.21^{a}	1.59^{ab}	2.41^{a}	2.09^{a}
D_4	$DORB_0$+E	1.45	1.41^{ab}	1.71^{a}	2.02^{a}	2.33^{a}	2.38^{b}	1.56^{ab}	2.23^{ab}	1.97^{a}
D_5	$DORB_5$+E	1.61	1.57^{a}	1.73^{a}	2.08^{a}	2.21^{ab}	2.78^{ab}	1.66^{a}	2.31^{a}	2.05^{a}
D_6	$DORB_{7.5}$+E	1.45	1.39^{ab}	1.62^{ab}	2.05^{a}	2.02^{abc}	3.26^{a}	1.52^{abc}	2.34^{a}	2.00^{a}
D_7	$FDORB_5$	1.53	1.21^{c}	1.47^{b}	1.66^{b}	1.94^{bc}	2.22^{b}	1.38^{c}	1.91^{c}	1.71^{c}
D_8	$FDORB_{7.5}$	1.31	1.27^{bc}	1.63^{ab}	2.10^{a}	1.83^{c}	2.47^{b}	1.45^{bc}	2.08^{bc}	1.83^{bc}
SEM		0.031	0.029	0.022	0.033	0.047	0.087	0.021	0.035	0.026
Stat. Significance		NS	P<0.01	P<0.05	P<0.01	P<0.01	P<0.01	P<0.05	P<0.01	P<0.01

DORB - De-oiled rice bran and FDORB – Fermented de-oiled rice bran
Mean values bearing different superscripts within a column differ significantly. NS- Non-significant (P>0.05).

The mortality (Table 4.4.4) occurred during feeding trail was well within the normal range and not found to be related to the fermentation DORB and their inclusion level.

Table 4.4.4: Livability of experimental birds (0-42 days)

Diet	Treatment	No. of chicks		Mortality	Livability
		Started	Survived	(%)	(%)
D_1	$DORB_0$	32	32	0.00	100.00
D_2	$DORB_5$	32	31	3.13	96.88
D_3	$DORB_{7.5}$	32	31	3.13	96.88
D_4	$DORB_0$+E	32	30	6.25	93.75
D_5	$DORB_5$+E	32	32	0.00	100.00
D_6	$DORB_{7.5}$+E	32	31	3.13	96.88
D_7	$FDORB_5$	32	30	6.25	93.75
D_8	$FDORB_{7.5}$	32	32	0.00	100.00

DORB - De-oiled rice bran and FDORB - Fermented de-oiled rice bran

The present study has clearly demonstrated that neither the fermentation nor the enzyme supplementation had any effect on gain in body weight by the experimental chicks up to 6 weeks of age. Nevertheless, feed intake and FCR values were also unaffected during early phase (0-3 weeks of age). However, during later phase (3-6 weeks of age), the chicks of D_7 group, in which FDORB was added @ 5% in feed, recorded significantly lower feed intake but improved FCR values than the groups of chicks offered untreated DORB, with or without enzymes supplementation in feed. Moreover, almost similar trend was maintained in feed intake and FCR values during overall phase (0-6 weeks of age), wherein, the chicks of D_7 group was found to have significantly ($P<0.01$) lower feed intake and better FCR values as compared to the chicks of other group containing different inclusion levels of raw DORB, with or without enzymes through feed. The feed intake and feed conversion ratio were

significantly improved in birds fed FDORB in comparison with their counterparts fed unprocessed DORB. The fermentation of DORB was worthy in improving the growth performance. Moreover, supplementation of enzymes to unprocessed DORB-based diets or addition of enzymes with 5 and 7.5% of untreated DORB did not further improve the growth performance of broilers.

To the best of the author's knowledge in published electronic data base there is no direct study involving feeding of fermented DORB with *Aspergillus niger* to broiler chicken. In the present, we demonstrated the effect of feeding of fermented DORB on growth performance of broiler chickens for the first time. Various scientific studies with other cereals, revealed similar findings to the present results. Manwar and Mandal (2009) fed sorghum with raw, soaked and reconstituted with or without enzyme supplementation to the broilers found that soaking and reconstitution of sorghum improved remarkably the FCR in initial phase (0-3 weeks); whereas feed intake was hardly affected. Moreover, said treatments to sorghum had significantly beneficial effect on body weight gain (0-6 weeks); whereas, results were comparable in present study. Adrizal *et al.* (1996) observed that feeding of enzyme treated DORB at graded levels up to 22.5% could not have any significant effect on feed intake, FCR and body weight gain in broiler chicken. Darwazeh (2010) suggested that inclusion of fermented WB with rumen filtrate up to 15% of broiler finisher diet did not have any adverse effect in the performance of broilers. The results of the present study are not in agreement with the other scientific studies in which no beneficial effect of the fermented palm kernel cake with rice bran substrate as component of ration on overall performance in broilers was demonstrated (Swe, 2004).

Albeit, water treatment of cereal based-diets showed beneficial effects in fast growing birds such as reduced *in vivo* viscosity, improved body weight, feed efficiency and increased ME intake (Yasar and Forbes, 1999; Bedford, 1995). Similarly, Tyagi *et al.* (1997) reported that the chicks fed on water-soaked wheat exhibited slightly higher weight gain than the chicks fed on untreated wheat based diet. It appears that the soaking of WB probably increased the susceptibility of starch to enzymatic degradation leading to improved performance of the chicks through better energy utilization. Adrizal *et al.* (1996) found that enzyme supplementation of DORB showed no significant improvement in the solubility or utilization of arabinoxylans or pentosans (NSPs) at gut level. The non-significant effect of soaking and fermentation on body weight gain may be explained by the aforesaid fact. Thus, it is inferred that the water soaking and fermentation of DORB improved the feed intake and FCR growing chicks, but could not affect the growth performance.

Protein and energy efficiency

The data on effect of dietary treatments on protein and energy efficiency in broilers have been presented in Table 4.4.5 and the analyses of variance of the same in Table 4.4.5a.

On cumulative basis, it is evident that there were statistically significant ($P<0.05$) differences in protein and energy efficiency of broiler chicks under different inclusion levels of either raw DORB or FDORB, with or without enzymes through feed during 0-3 (starting phase). During 3-6 (finishing phase) weeks of age, the values were again significantly ($P<0.01$) different amongst various treatments. Similar and significant ($P<0.01$) trends were observed in both the cumulative protein and energy conversion efficiencies as in FCR

during the 0-6 (overall phase) weeks of age. Among birds fed raw DORB with or without enzymes, overall protein and energy utilization efficiency became poorer with the inclusion level of raw DORB with or without enzymes in diets.

Table 4.4.5: Effect of feeding fermented de-oiled rice bran on protein efficiency (PE) and energy efficiency (kcal ME intake: gain, g) of broilers at different growth phases

Diet	Treatment	Protein efficiency			Energy efficiency		
		0-3 wk	3-6 wk	0-6 wk	0-3 wk	3-6 wk	0-6 wk
D_1	$DORB_0$	0.33^{abc}	0.42^{ab}	0.39^{ab}	4.30^{abc}	6.48^{ab}	5.65^{a}
D_2	$DORB_5$	0.34^{ab}	0.45^{a}	0.41^{a}	4.43^{ab}	6.88^{a}	5.96^{a}
D_3	$DORB_{7.5}$	0.34^{ab}	0.46^{a}	0.41^{a}	4.47^{ab}	6.98^{a}	6.00^{a}
D_4	$DORB_0$+E	0.34^{ab}	0.43^{ab}	0.39^{a}	4.40^{ab}	6.47^{ab}	5.68^{a}
D_5	$DORB_5$+E	0.36^{a}	0.44^{a}	0.41^{a}	4.65^{a}	6.71^{a}	5.90^{a}
D_6	$DORB_{7.5}$+E	0.32^{abc}	0.44^{a}	0.40^{a}	4.27^{abc}	6.77^{a}	5.76^{a}
D_7	$FDORB_5$	0.30^{c}	0.36^{c}	0.34^{c}	3.88^{c}	5.52^{c}	4.90^{b}
D_8	$FDORB_{7.5}$	0.31^{bc}	0.40^{bc}	0.36^{bc}	4.07^{ab}	6.03^{bc}	5.25^{b}
SEM		0.0045	0.0068	0.0052	0.0597	0.1019	0.0758
Stat. significance		P<0.05	P<0.01	P<0.01	P<0.05	P<0.01	P<0.01

DORB - De-oiled rice bran and FDORB – Fermented de-oiled rice bran
Mean values bearing different superscripts within a column differ significantly.
NS- Non-significant (P>0.05).

However, the birds fed FDORB @ 5 and 7.5% inclusion level had significantly (P<0.01) better overall protein and energy efficiency as compared to raw DORB with or without enzymes. Moreover, in birds fed on raw DORB with enzymes, the overall protein efficiency was statistically similar (P<0.01) to control group. Similarly, energy conversion in birds fed raw DORB with enzymes showed similar trend. The birds received fermented DORB consumed significantly (P<0.01) lower energy per unit gain than in raw DORB with or without enzymes fed group. The present results are in accordance with Manwar and Mandal (2009) who noted protein and energy efficiency in control

and reconstituted sorghum groups was better ($P<0.05$) as compared to unprocessed sorghum fed groups, during the starter phase (0-3 week). Also, the best efficiency for both protein and energy efficiency was found when birds fed reconstituted wheat (Manwar and Mandal, 2009). Arbinoxyloligosaccharides are oligosaccharides derived by partial hydrolysis of arbinoxylan (Courtin *et al.*, 2008), whereas uncleaved arabinoxylan hamper efficient feed utilization (Choct and Annison, 1992). It appears that on soaking of DORB during fermentation, possibly increased the availability of starch to enzymatic degradation leading to improve performance of the chicks through better energy utilization. Arabinoxylooligosaccharides derived from WB have been shown to have beneficial effects on the feed utilization efficiency (Courtin *et al.*, 2008). Likewise, it also present in DORB substrate which hydrolysis to arabinoxylooligosaccharides after soaking during fermentation, which could be attributed beneficial effects on feed efficiency. However, contrary to the present study results, cumulative protein and energy efficiency was unaffected due to dietary treatments during the 0-6 weeks period. In present study, protein utilization was better in birds fed FDORB in comparison with those fed untreated DORB. Same researchers opined that protein utilization efficiency was better in birds fed reconstituted sorghum in comparison with those fed untreated sorghum (Manwar and Mandal, 2009). In contrast to present results, Choct and Annison (1990) reported that though wheat pentosans decreased the protein utilization by increasing the endogenous amino acid secretions and as well as by inhibiting the true digestibility. Added pentosans from DORB based diets reduced digestibility and affect the performance of poultry. RB contains 10.05% pentosans (Houston, 1972). These NSPs are poorly digested by poultry (about 12%), resulting into reduced

energy utilization and interfere with the digestibility of other intrinsic feed components. Inclusion of high levels of wheat gave rise to higher *in vivo* viscosity (Allen *et al.*, 1997). Wyatt *et al.* (1999) reported that the diets supplemented with enzymes increased the energy availability by 3.3%. It has been reported that the enzymes improved the utilization of nutrients and improved feed efficiency by degrading the viscous polysaccharides present in wheat (Choct and Annison, 1991). Thus, the results of the present study clearly signify that the birds fed FDORB @ 5 and 7.5% inclusion level had better protein and energy efficiency as compared to the respective the raw counterparts.

4.4.2 Feed cost of broiler production

The data on feed cost of broiler production has been presented in Table 4.4.6 and the analyses of variance of the same in Table 4.4.6a (given as in Annexure).

The feed cost of production differed significantly ($P<0.01$) due to the dietary treatments. As formulating nutrient requirement recommended by BIS specification for broiler chickens, the cost of feed increased in diets containing raw DORB and enzymes, which resulted in increased feed cost of production. The feed cost per unit gain calculated in most of the dietary treatments was statistically ($P<0.01$) significant amongst the treatments groups. Birds fed diet containing FDORB @ 5% revealed statistically lower than the control and raw DORB with or without enzyme supplementation groups. The feed cost per unit gain noted higher in diet containing raw DROB, with or without enzyme supplementation than that of control groups. Similar trend was found for the feed cost per unit meat production. Fermentation of DORB decrease the cost of feed per kg gain or meat over raw DORB with or without enzymes fed groups, which was

statistically comparable to control group. However, enzyme addition was not beneficial in reducing the feed cost of broiler production.

Similarly Manwar and Mandal (2009) found that the cost of feed was reduced as the level of sorghum increased in the diet replacing maize as sorghum is cheaper than maize. The feed cost calculated on the basis of weight gain or meat yield remained similar ($P>0.05$) for all the diets. They further stated that differences in the price of maize and sorghum compensated the increase in the weight gain. However, in present study, the feed price reduced significantly ($P<0.05$) in FWB over corresponding raw groups with or without enzyme supplementation.

The addition of enzymes was also not beneficial in terms of economics of broiler production in raw wheat bran based diets. However, the feed cost per kg body weight gain or meat yield was lower in FDORB fed groups. Similar trend was observed by Manwar and Mandal (2009). It is inferred that, the feed cost per unit weight gain or meat yield apparently reduced due to fermentation.

Table 4.4.6: Effect of feeding fermented de-oiled rice bran on feed cost (Rs.) of broiler production

Diet	Treatment	Feed cost /kg gain			Feed Cost /kg BWt	Cost/kg meat
		0-3 wk	3-6 wk	0-6 wk		
D_1	$DORB_0$	24.54^{bcd}	34.92^{bc}	30.94^{cd}	30.00^{cd}	41.36^{bc}
D_2	$DORB_5$	25.94^{abc}	38.26^{ab}	33.64^{a}	32.63^{a}	44.37^{ab}
D_3	$DORB_{7.5}$	26.63^{ab}	39.34^{a}	34.37^{a}	33.28^{a}	45.19^{a}
D_4	$DORB_0$+E	25.33^{abc}	34.91^{bc}	31.23^{bcd}	30.29^{bcd}	41.57^{bc}
D_5	$DORB_5$+E	27.52^{a}	37.29^{ab}	33.44^{ab}	32.41^{ab}	44.32^{ab}
D_6	$DORB_{7.5}$+E	25.66^{abc}	38.18^{ab}	33.10^{abc}	32.11^{abc}	43.34^{ab}
D_7	$FDORB_5$	22.45^{d}	30.02^{d}	27.15^{e}	26.35^{e}	36.04^{d}
D_8	$FDORB_{7.5}$	23.61^{cd}	33.20^{c}	29.39^{d}	28.49^{d}	38.90^{c}
SEM		0.377	0.624	0.475	0.458	0.606
Stat. significance		P<0.01	P<0.01	P<0.01	P<0.01	P<0.01

DORB - De-oiled rice bran, FDORB – Fermented De-oiled rice bran, BWT- Final body weight.
Mean values bearing different superscripts within a column differ significantly.
NS- Non-significant (P>0.05).

4.4.3 Nutrient utilization

The data on N-retention, N-intake, excreta dry matter, gross energy and dry matter metabolisability, AME values (both classical and nitrogen corrected), Ca-retention and P-retention have been presented in Table 4.4.7 and the analyses of variance of the same in Table 4.4.7a.

The N-retained (g/b) was significantly ($P<0.05$) improved due to fermentation though the nitrogen retention in group fed on FDORB was statistically non significant in comparison to untreated DORB, with or without enzymes fed group. The percent N-retention amongst different treatments were statistically non-significant. However, the DM metabolizabilty and gross energy metabolizability differed ($P<0.05$) due to the dietary treatments. When birds fed diets containing untreated and enzyme supplemented DORB, the AMEn values was comparable with control group. The improvement in Ca-retention and P-retention were significant ($P<0.05$) in diets containing FDORB. The Ca and P retention were statistically ($P<0.01$) higher in birds fed FDORB at 5 and 7.5 % inclusion level than the birds fed control and raw DORB, with or without enzyme supplementation in broiler ration.

The present findings are in accordance with Manwar and Mandal (2009) found that when different sorghum types were compared, the gross energy metabolizability and the AMEn values improved ($P<0.01$) due to reconstitution with or without enzymes compared to all other types of grains. The per cent excreta dry matter was significantly increased ($P<0.01$) due to enzyme supplementation to raw sorghum grains as well as due to addition of enzymes during the process of reconstitution over their respective counterparts. Gross energy metabolizability was significantly higher in diets containing

Table 4.4.7: Effect of feeding fermented de-oiled rice bran on nutrient utilization by broilers

Diet	Treatment	N-retd. (g/b/d)	N-ret.(%) (%)	ExcretaDM (%)	DMM (%)	GEM (%)	AMEn	Difference (%)	Ca-ret. (%)	P-ret. (%)
D_1	$DORB_0$	1.47^{ab}	54.82	58.49^{a}	72.12^{a}	74.18^{a}	2923	0.61	31.44^{bc}	40.33^{d}
D_2	$DORB_5$	1.47^{ab}	55.56	54.24^{ab}	71.63^{a}	73.29^{ab}	2937	1.11	31.22^{cd}	41.71^{cd}
D_3	$DORB_{7.5}$	1.65^{a}	57.52	51.46^{ab}	72.68^{a}	72.94^{b}	2954	1.84	29.23^{d}	41.10^{cd}
D_4	$DORB_0$+E	1.49^{ab}	50.92	58.65^{a}	67.20^{c}	69.90^{d}	2942	1.17	31.61^{bc}	42.35^{cd}
D_5	$DORB_5$+E	1.70^{a}	56.23	52.88^{ab}	68.64^{b}	70.94^{c}	2938	1.28	33.47^{b}	43.33^{bc}
D_6	$DORB_{7.5}$+E	1.65^{a}	54.26	47.07^{bc}	68.98^{b}	73.36^{ab}	2950	1.70	33.40^{b}	44.90^{b}
D_7	$FDORB_5$	1.36^{b}	51.89	51.37^{ab}	68.09^{bc}	71.36^{c}	2961	2.04	36.00^{a}	49.94^{a}
D_8	$FDORB_{7.5}$	1.32^{b}	48.89	42.89^{c}	68.85^{b}	71.01^{c}	2933	1.13	35.77^{a}	49.76^{a}
SEM		0.03	0.91	1.22	0.36	0.27	4.46	0.15	0.423	0.654
Stat. significance		P<0.05	NS	P<0.01	P<0.01	P<0.01	NS	NS	P<0.01	P<0.01

DORB - De-oiled rice bran and FDORB – Fermented de-oiled rice bran
Mean values bearing different superscripts within a column differ significantly. NS- Non-significant (P>0.05).

wheat reconstituted with enzymes compared to their untreated counterparts. AMEn values in contrary to the present findings were significantly higher in diets containing wheat reconstituted with enzymes compared to their untreated counterparts. McCracken and Quintin (2000) observed no significant effect of wheat or enzyme addition on AME contents of the diet. However, wheat processed or unprocessed when incorporated at 100% replacement level of maize tend to decrease the dry matter metabolizability, nitrogen retained per bird and AME values (both classical and nitrogen corrected). The NSPs in DORB exhibit anti-nutritive activity when present in poultry diet. The high levels of arabinoxylan (pentosans) in DORB were responsible for low metabolizable energy value and affects MEn values of chickens (Veldman and Vahl, 1994). Water-extractable and alkali-extractable fractions when added to a commercial type broiler diet to provide 25.9-65.7 g arabinoxylan/kg, a significant dose-dependent depression in AME value of the diet occurred and at highest levels of inclusion the ileal digestibility of starch, protein and lipid were reduced from 96% to 82%, 75% to 61% and 93% to 69%, respectively (Mollah *et al.*, 1983). Rotter *et al.* (1989), Pettersson and Aman (1989), and Viveros *et al*. (1994) have shown that supplementation of diet containing NSP with fiber- degrading enzymes improves AMEn values of diets.

The present study revealed a significant ($P<0.01$) improvement in Ca and P retention in diets containing FDORB over the raw counterparts and control. It is attributed due to release of microbial phytase by *A. niger* and finally improving the P retention. Supplementation of diets with feed enzymes and phytase also has been effective in improving the utilization of PP in poultry (Farrel *et al.*, 1993). However, Dinani (2009) studied certain parameters in quails wherein, nutrient utilization indicated that 15% TGM without

enzyme showed comparatively poor nutrient utilization in terms of DM, energy, AME metabolizability, N, Ca and P retention as compared to other dietary treatments. Further, enzyme supplemented groups showed better nutrient utilization as compared to unsupplemented groups and FTGM showed better nutrient utilization as compared to enzyme supplemented groups. It is inferred that, diet containing FDORB found beneficial effect for nutrient utilization and improved the Ca and P retention due to fermentation.

4.4.4 Carcass traits

The influence of feeding fermented de-oiled rice bran to broilers chickens up to 6 weeks of age on various carcass traits (pre-slaughter fasting live weight, dressing percentage, blood loss, feather loss, eviscerated weight, giblet yield, ready-to-cook-yield and abdominal fat), yield of cut-of-parts (breast, drumsticks, thighs, back, neck and wings) and organs weights (gizzard, heart, liver, spleen and bursa) of broilers (% live weight) at 6 weeks of age were studied and the results are presented hereunder.

Slaughter traits

The data on effect of dietary treatments on blood and feather loss, eviscerated yield, edible yield, giblet yield, ready-to-cook and abdominal fat pad (% live weight) of broilers have been presented in Table 4.4.8 and the analyses of variance of the same in Tables 4.4.8a. The analysis of variance for slaughter traits of broilers revealed a non significant influence of treatments on blood loss, eviscerated yield, edible yield, giblet yield and ready-to-cook (eviscerated with giblet weight) yield. Similarly, the abdominal fat pad (% live weight) also showed no significant difference due to different inclusion levels of raw DORB, with or without enzymes and FDORB than control

Table 4.4.8: Effect of feeding fermented de-oiled rice bran on carcass traits of broilers (% live weight) at 6 weeks of age

Diet	Treatment	Bloodloss	Featherloss	Eviscerated yield	Edibleyield	Gibletyield	Ready-to-cook	Abd.fat pad
D_1	$DORB_0$	4.30	6.50^{ab}	67.64	73.45	4.91	72.55	1.06
D_2	$DORB_5$	3.85	5.11^{c}	68.49	74.81	5.04	73.54	1.15
D_3	$DORB_{7.5}$	4.39	7.15^{a}	69.06	74.31	4.58	73.65	0.94
D_4	$DORB_0$+E	4.25	7.25^{a}	67.83	74.04	5.03	72.86	1.19
D_5	$DORB_5$+E	3.89	7.27^{a}	68.09	74.36	5.04	73.13	1.09
D_6	$DORB_{7.5}$+E	4.73	5.32^{bc}	69.01	75.27	5.07	74.08	1.19
D_7	$FDORB_5$	3.73	6.04^{abc}	68.36	74.34	4.75	73.11	0.92
D_8	$FDORB_{7.5}$	4.06	6.25^{abc}	68.22	74.36	5.00	73.23	1.35
SEM		0.121	0.174	0.264	0.256	0.056	0.260	0.042
Stat. significance		NS	P<0.01	NS	NS	NS	NS	NS

DORB - De-oiled rice bran and FDORB – Fermented de-oiled rice bran
Mean values bearing different superscripts within a column differ significantly.
NS- Non-significant (P>0.05).

birds. However, feather loss was significantly ($P<0.01$) different amongst various treatments. These finding differ from other researchers, Manwar (2007) who found that inclusion of wheat and its processed forms in broiler diets could not exert any significant influence on per cent blood loss, eviscerated yield, edible yield and abdominal fat pad. The results pertaining to carcass characteristics were similar to findings of Rama Rao *et al.* (2002), Tyagi *et al.* (2003) and Sannamani (2002) who reported that feeding of sorghum did not affect blood loss. Also, blood loss did not differ significantly ($P>0.05$) between treatments groups in broiler quail when fed with fermented TGM with or without enzymes supplementation (Dinani, 2009). Dressing and eviscerated per cent in contrary to the present findings were significantly higher ($P<0.05$) in FTGM and their corresponding counterparts with enzyme supplementations as compared to control (Dinani, 2009). However, per cent giblet yield was lower ($P<0.01$) in groups fed on diets containing 75 or 100 % reconstituted wheat in comparison to control group (Manwar, 2007).

In present study the feather loss was relatively higher in FDORB group. No definite trend was followed in terms of feather loss per cent. Contrary to this, feeding of sorghum did not affect feather loss (Manwar, 2007). However, the eviscerated yield, edible yield and ready-to-cook (eviscerated with giblet weight) were statistically similar in all dietary treatments in present study. In accordance to this, Manwar (2007) observed that feeding of sorghum did not affect eviscerated, edible and ready-to-cook yield. Bhutia (2006) reported that inclusion level of TGM up to 10% in broiler quail ration with or without enzyme did not significantly ($P<0.05$) effect the dressed and eviscerated weight.

Cut-up parts

The relative mean values of cut-up parts of carcass (% live weight) measured at the end of 6th weeks of age of broilers under different treatments are given in Table 4.4.9 and the analyses of variance of the same in Tables 4.4.9a.

The results revealed that the weights of breast, drumsticks, thighs, back, neck and wings did not differ significantly (P>0.05) amongst treatments. The relative breast, drumsticks, thighs, back, neck and wings weights influenced by different inclusion levels of raw DORB, with or without enzymes and FDORB were found to be similar in all treatment groups.

Table 4.4.9: Effect of feeding fermented de-oiled rice bran on cut-up parts (% eviscerated weight) in broilers at 6 weeks of age

Diet	Treatment	Breast	Drumstick	Thigh	Back	Neck	Wings
D_1	$DORB_0$	24.80	15.58	15.05	23.59	6.15	14.21
D_2	$DORB_5$	24.75	15.35	14.46	24.30	6.16	13.67
D_3	$DORB_{7.5}$	26.32	15.35	14.30	23.58	5.93	13.40
D_4	$DORB_0$+E	24.77	16.04	14.40	24.50	5.79	13.39
D_5	$DORB_5$+E	25.41	15.04	15.10	24.08	6.34	13.27
D_6	$DORB_{7.5}$+E	25.20	15.64	14.24	23.96	6.45	13.48
D_7	$FDORB_5$	24.51	16.44	14.72	24.10	5.89	13.51
D_8	$FDORB_{7.5}$	24.90	16.21	14.55	23.79	6.04	13.76
SEM		0.197	0.135	0.136	0.165	0.082	0.085
Stat. significance		NS	NS	NS	NS	NS	NS

DORB - De-oiled rice bran and FDORB – Fermented de-oiled rice bran
Mean values bearing different superscripts within a column differ significantly. NS- Non-significant (P>0.05).

The results of some indirect studies indicated that inclusion of wheat either raw or processed did not alter above said cut-up part traits in broilers (Manwar, 2007). In this study aforesaid cut-up parts remained statistically similar amongst various dietary treatments.

Similar result was observed in feeding reconstituted sorghum group in broiler chickens (Manwar, 2007). However, the present results on breast yield and wings weights are contrary to same author when birds fed diets containing reconstituted sorghum.

Organ weights

The results on the effect of feeding FDORB to broilers chickens up to 6 weeks of age on vital organs and immune organs (per cent live weight) are presented in Table 4.4.10. The analysis of variance of gizzard, heart and liver as per cent live weight showed a non significant difference (P>0.05) due to different inclusion levels of raw DORB, with or without enzymes and FDORB (Table 4.4.10a)

Table 4.4.10: Effect of feeding fermented de-oiled rice bran on vital organs and immune organs (% live weight) of broilers at 6 weeks of age

Diet	Treatment	Gizzard	Heart	Liver	Spleen	Bursa
D_1	$DORB_0$	2.14	0.46	2.30	0.18	0.13
D_2	$DORB_5$	2.15	0.47	2.43	0.23	0.17
D_3	$DORB_{7.5}$	2.01	0.47	2.11	0.20	0.19
D_4	$DORB_0$+E	2.17	0.50	2.37	0.22	0.20
D_5	$DORB_5$+E	2.35	0.48	2.20	0.19	0.13
D_6	$DORB_{7.5}$+E	2.28	0.45	2.34	0.19	0.15
D_7	$FDORB_5$	2.08	0.47	2.20	0.21	0.12
D_8	$FDORB_{7.5}$	2.16	0.46	2.38	0.21	0.20
SEM		0.038	0.008	0.033	0.007	0.011
Stat. significance		NS	NS	NS	NS	NS

DORB - De-oiled rice bran and FDORB – Fermented de-oiled rice bran
Mean values bearing different superscripts within a column differ significantly.
NS- Non-significant (P>0.05).

Likewise, the mean weights of spleen and bursa weights as per cent live noted a non significant difference in all dietary treatments. The results pertaining to organ weights are similar to the earlier reports (Rama Rao *et al.*, 2002; Tyagi *et al.*, 2003; Manwar,

2007). Feeding of FDORB at different inclusion level did not affect heart, liver and gizzard weight of birds fed on diets containing raw DORB. In accordance to Manwar (2007) reported that the mean weight of heart, liver and bursa did not significantly differ due to inclusion of raw and reconstituted wheat in broiler diet. Similarly, Bhutia (2006) reported gizzard, heart, spleen weight did not differ significantly ($P>0.05$) at 0, 5 and 7.5% TGM levels with or without enzyme supplementation in broiler quail ration. Bakshi *et al.* (1964), Prakash and Singh (1984) and Nagra (1984) reported that liver weight were found to be unaffected by the feeding of guar meal to chicks. However, the lower relative gizzard and spleen weight was recorded in group fed raw wheat at 50% inclusion level of maize and reconstituted wheat but no definite trend was observed (Manwar, 2007). Moreover, Sannamani (2002) reported significantly higher weight of gizzard in broilers fed red sorghum based diet which was attributed to lower dietary energy level on inclusion of sorghum in diet as the red sorghum contains much less than in its white variety (Mandal *et al.*, 2006). Nelson *et al.* (1975) also reported the higher weight of gizzard and it might be due to higher amount of crude fiber. However, this change was not appeared in FDORB fed groups at both 5 and 7.5% inclusion level (Table 4.4.10). It was reported that most of the digestive organ weights were higher in broilers fed with sorghum based diets (Dixit and Baghel, 1998). It was also reported that the liver, gizzard, giblet and spleen weights were affected significantly by feeding of sorghum in broiler chicks (Attia and Rahman, 1996).

4.4.5 Serum and immune parameters

The data pertaining to effect of dietary treatments on serum total cholesterol, triglycerides and uric acid, cell mediated immune

response to PHA-P and humoral immune response to SRBC are presented in Table 4.4.11 and the analyses of variance of the same in Tables 4.4.11a (given as in annexure).

Table 4.4.11: Effect of fermented de-oiled rice bran on certain serum (mg/100ml), immune parameters and ceaca microbial count (cfu/g) of broilers

Diet	Treatment	Cholesterol	Triglycerides	Uric acid	CMI response (mm)	HA titer (log 2)	Caeca microbes TPC (cfu/g)
D_1	$DORB_0$	65.45	82.45^{ab}	4.59^{c}	0.34^{c}	6.57	59.50^{a}
D_2	$DORB_5$	61.68	82.23^{ab}	4.36^{c}	0.37^{bc}	7.57	43.83^{b}
D_3	$DORB_{7.5}$	68.96	76.38^{bc}	4.63^{bc}	0.40^{ab}	7.70	43.17^{b}
D_4	$DORB_0$+E	72.73	89.04^{a}	4.67^{bc}	0.42^{a}	7.45	44.50^{b}
D_5	$DORB_5$+E	75.00	77.24^{bc}	4.88^{abc}	0.42^{a}	7.95	42.67^{b}
D_6	$DORB_{7.5}$+E	70.67	75.96^{bc}	5.30^{ab}	0.38^{bc}	7.82	37.67^{b}
D_7	$FDORB_5$	78.16	71.81^{c}	4.94^{abc}	0.41^{ab}	8.70	33.33^{b}
D_8	$FDORB_{7.5}$	69.37	79.05^{bc}	5.44^{a}	0.42^{a}	7.95	33.17^{b}
SEM		1.815	1.222	0.085	0.006	0.186	2.168
Stat. significance		NS	P<0.05	P<0.05	P<0.01	NS	P<0.05

DORB - De-oiled rice bran and FDORB – Fermented de-oiled rice bran
Mean values bearing different superscripts within a column differ significantly.
NS- Non-significant (P>0.05).

The level of serum cholesterol was found to remain unaffected by the various inclusion levels of raw DORB, with or without enzymes and FDORB. The level of serum cholesterol of FDORB group remained statistically non-significant as compared to their raw DORB fed counterparts. However, the serum triglycerides values were significantly lower in D_3, D_5, D_6, D_7 and D_8 than D_1, D_2 and D_4. When the types of DORB compared, the birds fed diets containing FDORB with or without enzymes showed increased (P<0.01) immune response measured in terms of cell mediated immune response to PHA-P compared to control group. Compared to the control group, there were no statistical significant differences in terms of humoral response to SRBC in all

the groups. The serum uric acid values were significantly ($P<0.05$) different amongst treatment. The serum uric acid value of D_8 (FDORB7.5) group was significantly ($P<0.05$) higher than D_1 (control) group. Whereas, compared to the control group, there were statistical significant ($P<0.05$) differences in triglycerides values in all dietary treatment groups. It was observed that There are hardly any studies in the literature involving effect of feeding of fermented DORB with *A. niger* to broiler chicken, in regards to serum biochemical and immune response have been carried out earlier. Present results are similar to the earlier reports, Manwar and Mandal (2009), who worked on reconstituted sorghum, and opined that feeding processed sorghum did not influence the serum cholesterol of broilers. Similarly, triglycerides and uric acid concentration also unaffected due to feeding reconstituted sorghum, which were contrary to present findings. However, the present results on PHA-P and CMI response of birds are also similar to Manwar (2007) wherein PHA-P was better in sorghum-fed groups. Moreover, humoral response to SRBC did not differ due to inclusion of reconstituted sorghum and their raw counterparts in broiler diets. However, same authors suggested further that when the types of wheat compared, the birds fed diets containing wheat reconstituted with or without enzymes showed increased ($P<0.01$) immune response measured in terms of humoral response to SRBC compared to their counterparts fed raw or supplemented with enzymes.

In the present study, compared to the control group, there were no statistical significant differences in serum cholesterol in the dietary treatment groups. The highest triglycerides concentration was recorded in group fed control with enzyme supplemented ($DORB_0$+E). Lowest triglycerides concentration was observed in group

fed FDORB compared to birds in other groups. The birds fed on diets containing FDORB showed higher serum uric acid as compared to their counterparts fed raw DORB and control. But no specific trend was observed due to fermentation.

The immune response in terms of humoral response to SRBC considered in the present study did not show any significant ($P>0.05$) change in the dietary treatments. In general, birds fed on DORB, as such or fermented diets had a similar immune response in terms of CMI response and HA titer to the birds fed DORB based diets. However, on comparing the different types of DORB, it was revealed that the birds fed diets containing FDORB and raw DORB with or without enzymes showed improved ($P<0.01$) immune response measured in terms of cell-mediated immune response to phytohaemagglutinin-P compared to their counterparts fed raw or supplemented with enzymes. Thus it signifies the superior cellular immune response in broiler chickens can be achieved by feeding of fermented DORB.

4.4.6 Caeca microbial count

The data on mean total microbial counts of caeca contents (cfu/g) of broiler as influenced by dietary levels of DORB, with or without enzyme supplementation and FDORB have been presented in Table 4.4.11; along with their analysis of variance in Table 4.4.11a.

Caeca microbial count ranged from 59.50 in control diet (D_1) to 33.17 in 7.5% FDORB (D_8) group. Caeca microbial count was significantly ($P<0.05$) higher in control (D_1) as compared to other groups. Enzyme supplemented groups along with DORB (D_4, D_5, D_6) significantly ($P<0.05$) reduced caeca microbial count as compared to

their respective non-enzyme supplemented groups (D_1, D_2 and D_3). FDORB at 5 and 7.5% (D_7 and D_8) reduced caeca microbes significantly ($P<0.05$) as compare to control and other dietary groups. The present study are in line with Bailey *et al.* (1991), Ishihara et al. (2000), Lee *et al.* (2003b), Gujral (2005), Bhutia (2006) and Dinani (2009). Bailey *et al.* (1991) reported that chicken fed fructo-oligo-saccarides (FOS) had four fold reduction in the level of Salmonella present in the caeca. Ishihara *et al.* (2000) who reported that partially hydrolyzed guar gum prevented colonization of *Salmonella enteriditis* in young and laying hen. Gujral (2005) reported that the fructo-oligo-saccharides (FOS) and mannan oligo-saccharides (MOS) reduced significantly ($P<0.05$) with caecal colonization of *Salmonella typhimurium* upon challenge with live bacteria on third day of age in broiler quails. Eeckhaut *et al*. (2008) reported that arabinoxylooligo-saccharides released from DORB with or without enzyme supplementation or from partial hydrolysis of arainoxylan (NSP) of DORB during soaking or fermentation significantly ($P<0.05$) reduced caeca microbial count of Salmonella sp. as compared to control in broiler chicken. They further deduced that enhanced the population of beneficial bacteria like Bifidobacteria that consequently would have lead to reduction in caeca count of Salmonella in broilers.

The reduction in the colonization of total microbial load in the present study was possibly the effect of arabinoxylooligosaccharides hydrolysed from the substrate DORB, acting as a prebiotic for beneficial microbes and thus preventing the colonization of pathogens by competitive exclusion and increased microbial fermentation in caeca.

4.4.7 Sensory evaluation

The data on various sensory characteristics of broiler meat as influenced by dietary treatments have been presented in Table 4.2.12

and the analysis of variance of the same in Table 4.4.12a. The appearance, flavour, juiciness, tenderness and overall acceptability score of fresh cooked meat as judged by the panelists was not found to be significantly (P>0.05) influenced by the various dietary treatments.

The appearance score was comparable amongst various treatments of DORB (P>0.05). It showed that inclusion of raw DORB, with or without feed enzyme and FDORB in broiler diets did not bring out much change in order to change the sensory quality attributes of meat. It also indicates that de-oiled rice bran NSPs may not be always altering the sensory quality of the meat. The present results are in agreement with Manwar (2007). However, it was reported that the sorghum-based diets did alter the pigmentation of skin in broilers (Sharda and Thakur, 1977).

Table 4.4.12: Effect of feeding fermented de-oiled rice bran on sensory attributes of meat

Diet	Treatment	Appearance	Texture	Juiciness	Flavour	Tenderness	Accept.
D_1	$DORB_0$	6.33	5.33	6.00	6.67	6.00	6.67
D_2	$DORB_5$	7.00	6.67	5.67	6.33	5.67	6.00
D_3	$DORB_{7.5}$	7.17	6.33	5.33	6.00	6.33	6.17
D_4	$DORB_0$+E	7.17	6.67	5.67	4.67	5.67	6.00
D_5	$DORB_5$+E	5.33	6.83	5.67	5.67	5.67	6.33
D_6	$DORB_{7.5}$+E	6.67	6.33	7.33	6.33	6.00	6.67
D_7	$FDORB_5$	6.33	6.00	7.33	6.00	6.33	6.67
D_8	$FDORB_{7.5}$	6.33	6.00	7.33	6.00	6.33	6.67
SEM		0.223	0.243	0.266	0.175	0.159	0.162
Stat. significance		NS	NS	NS	NS	NS	NS

DORB - De-oiled rice bran and FDORB – Fermented de-oiled rice bran
Mean values bearing different superscripts within a column differ significantly. NS- Non-significant (P>0.05).

Contrary to present study, in some findings which were reported that presence of NSPs may affects a general meat discolouration. Also, at levels more than 2% of tannin (tannic acid) affects egg production and egg weight, increases the frequency and degree of yolk mottling and causes a general yolk discoloration, but it does not seem to have any effect on albumen quality as measured by Haugh unit.

❖❖❖

Summary and Conclusions...

Summary and Conclusion

Poultry is the fastest growing agriculture sector and will grow at a faster rate in the coming years to meet the challenges in food production and food security, employment opportunities, alleviation of poverty, etc. The rising cost and scarcity of feed is a matter of great concern in poultry production. Scope exits to use alternate feed ingredients, which can be suitably incorporated in rations to make poultry production cheaper. Dependence on maize as a major ingredient of poultry feed is ever increasing. Wheat (*Triticum aestivum*) is the second most important cereal crop in India after rice, grown under diverse agro-climatic condition. India is fourth largest wheat producer in the world. Rice (*Oryza sativa*) is the principle cereals food in many part of the world. Wheat bran and rice bran are the main by-products available from their milling. This situation makes it necessary to undertake research work to evaluate and explore the usefulness wheat bran and de-oiled rice bran and thus channelize these cereal byproducts towards poultry feed formulation. The antinutritional factors such as non-starch polysaccharides (NSPs) and phytates are present in these brans. Poultry do not produce enzymes for hydrolysis of NSPs present in the cell wall of the bran, resulting in low feed efficiency. It has been established that the processing of feed brings about improved starch availability resulting in increased feed efficiency and protein utilization. Feed processing includes different kind of treatments applied for nutritional

improvement. Some of them are simple and some require expensive mechanization. The method of feed processing is selected on the basis of the nature of feed, its availability, its chemical composition, and presence of toxic factors, economic implications, the quantity to be processed and use of processed feed ingredients. *Aspergillus niger* is considered as generally recognized as safe (GRAS) microorganism as per WHO and FAO. It is a filamentous fungus and most preferred organism in the industrial production of fermented foods, organic acids and enzymes. Solid-substrate fermentation (SSF) systems have generated much interest in recent years because of several economical and practical advantages. SSF systems have also been reported to be an effective way to produce enzymes. SSF processes can be defined as "the growth of microorganisms (mainly fungi) on moist solid materials in the absence of free-flowing water". SSF of bran can increase the nutrients availability and improve their sensory characteristics, thus, adding value to these materials and creating new opportunities for their utilization. Fermentative process, especially SSF had been used to produce value added products from raw materials. Solid substrate fermentation is an anaerobic process in which the various intrinsic enzymes of bran origin are activated resulting into release of nutrients and their increased availability. It results in disruption of the protein matrix of the bran, release of enzymes, breakdown of cellular carbohydrates, and alteration in structure of intracellular starch and removal or inactivation of toxic substances. The benefits of addition of commercial enzyme preparation in mixed feed have not always been consistent. Therefore, in this investigation four experiments were carried out to augment availability of feed energy and nutrients, which not only help in saving feed but also help to reduce environmental pollution and bad aroma

in poultry house. Therefore, the agriculture products and by-products should undergo some processing methodologies *viz* SSF in order to make them suitable for poultry. And to study the effects of fermentation of WB and DORB with *A. niger* and optimum condition for its better growth on wheat and rice bran. Fermented WB and DORB, with or without enzymes on nutrient availability (*in vitro)* and on growth performance and immune response (*in vivo)* in broiler chickens. In experiment 1, *In- vitro* trials were conducted to optimize the conditions for growth of *A. niger* on WB and DORB. The WB and DORB were treated separately in laboratory scale by maintaining three different levels of moisture at constant temperature (37°C) and duration (72 h) i.e. moisture level 1: WB or DORB and water to soak at the ratio of 70 and 30 (w/v); moisture level 2: WB or DORB and water at the ratio 60 and 40 (w/v) and moisture level 3: WB or DORB and water at the ratio 50 and 50 (w/v). WB and DORB were soaked in tap water, mixed thoroughly and autoclaved at 15 psi for 15 min. Then, it was spread uniformly in a tray of 1.5 to 2.0 cm thick layer. Spore suspension was inoculated at the rate of one lac spore per kg of autoclaved WB and DORB. The polythene sheet (300 gauge) was spread over the tray after inoculation and spraying of spore suspension. The polythene sheet was folded on all the sides of the tray to create anaerobic condition for fermentation in BOD incubator. The best moisture level standardized in the earlier experiment was maintained for final fermentation. Subsequently, the brans were oven-dried at 60°C for 24 h. The fermented bran was then (with or without enzyme) tried on day-old broiler chicks up to their market age to find out their feeding value and improvement in feeding value, if any. They were compared simultaneously with the untreated bran at the same levels.

The commercial multi-enzyme preparation (*Brozyme*) was analyzed for different enzyme activities *viz*. α-amylase (EC 3.2.1.1) 2000 ± 51 mIU/g; β-glucanase (EC 3.2.1.21) 150 ± 25 mIU/g; xylanase (EC 3.2.1.8) 3000 ± 48 mIU/g; carboxymethyl cellulase (EC 3.2.1.4) 40 ± 12.5 mIU/g; pectinase 150 ± 48 mIU/g; proteinase 600 ± 52 mIU/g; α-galactosidase 250 ± 38 mIU/g; β-galactosidase (EC 3.2.1.37) 200 ± 21 mIU/g; lipase 400 ± 45 mIU/g and phytase 50± 4.8 mIU/g. The activities (mIU/kg) of different enzymes in fermented bran were - glucosidase 17566; β-D-xylosidase 49233; xylanase 1101; carboxymethyl cellulase 953; FTPase (filter paper degrading activity) 193 and α-amylase 2889.

In experiment 2, raw and the fermented WB and/or DORB types were subjected to *in vitro* studies for evaluation of nutrient availability in terms of metabolizable energy (AMEn), dry matter and energy metabolizability, *in vitro* pepsin-pancreatin digestibility (IVPPD), available carbohydrates, acid detergent fiber and available phosphorus. In experiment 3 and 4, feeding trials were conducted to study the effect of fermentation of WB and DORB, with or without enzymes on the production performance and immunocompetence in broiler chickens, respectively.

In experiment 3 and 4, 256 straight run commercial broiler chicks (*CARIBRO-Vishal* and *CARI-coloured*, respectively) were randomly allotted to eight dietary treatment groups, separately. In experiment 3 and 4, eight dietary treatments were formulated as D_1: Maize + soybean meal (control diet), D_2: wheat bran or de-oiled rice bran (5%) as such, D_3: wheat bran or de-oiled rice bran (7.5%) as such, D_4: wheat bran or de-oiled rice bran (0%) as such with multienzyme @ 0.5 g/kg, D_5: wheat bran or de-oiled rice bran (5%)

as such with multienzyme @ 0.5 g/kg, D_6: wheat bran or de-oiled rice bran (7.5 %) as such with enzyme @ 0.5 g/kg, D_7: fermented wheat bran or fermented de-oiled rice bran (5%) as sole bran and D_8: fermented wheat bran or fermented de-oiled rice bran (7.5%) as sole bran for 0-3 wk (starter mash) and 3-6 wk (finisher mash) growth phases, respectively. Each diet was offered to 32 broilers divided into four groups (replicates).

Body weight of individual broiler chick and feed intake of chicks allotted in replicates was recorded weekly up to 6 weeks of age. The mortality of birds was recorded as and when occurred, weighed and sent for postmortem examination. The feed conversion ratio (feed consumed to unit body weight gain), energy efficiency (kcal ME intake: gain in g) and protein efficiency (CP intake in g: gain in g) were calculated for each replicate. A metabolism trial (total collection method) of three days collection period was conducted at fourth week of age. At the end of 6th week of age, two birds from each replicate (one male and one female) were selected randomly and sacrificed for evaluation of carcass traits *viz*. relative weight of eviscerated carcass, cut-up parts, abdominal fat, vital organs (gizzard, heart and liver), immune organs (spleen and bursa). The organ weights and carcass traits were expressed as per cent of live weight. The prevailing market price of feed ingredients, supplements, enzymes, live weight gain and broiler meat were considered for calculation of feed cost per unit gain and meat yield. On 42nd day of experimental period, 3 ml blood was collected from two birds from each replicate selected randomly and serum was separated. The serum cholesterol, triglycerides and uric acid concentration was estimated. The cellular immune response was assessed by cutaneous basophilic hypersensitivity test *in vivo* by using PHA-P (phytohaemagglutinin, lectin from *Phasealus vulgaris)* on 22nd day post-hatch in eight birds from each

treatment group. On 37th day post-hatch, two birds per replicate were selected randomly and 1.0 ml suspension of sheep red blood cells (SRBC) was injected intravenously to study the primary antibody response to SRBC. On 35th day, two chicks per replicate per dietary treatment were sacrificed by cervical dislocation and caeca contents were collected in sterile vials for evaluation of total microbial load colonization as caeca microbial status. The sensory test was also conducted for the evaluation of the sensory attributes of broiler meat as influenced by the dietary treatments. Data obtained in all the four experiments were analyzed using standard statistical tools to study the treatment effects. The means of different dietary treatments were tested for statistical significance using Duncan's multiple range tests.

The overall results of the study are summarized as under:

- Chemical analysis showed that the DM loss increased gradually with the increase in moisture level in WB substrate. Amongst the all fermented groups, lowest DM loss (16.98%) was noted in water soaked WB at the ratio of 70:30 (w/v) and highest DM loss of 20.48% was found in water soaked WB at the ratio of 50:50 (w/v).
- As moisture level in the substrate (DORB) increased, the DM loss decreased gradually. Amongst all fermented groups, lowest DM loss (21.13%) was noted in FDORB soaked with water at the ratio of 50:50 (w/v), whereas; the highest DM loss (25.41%) was observed in FDORB soaked with water at the ratio of 70:30 (w/v).
- Fungal fermentation of WB increased CP from 14.89 to 19.78%, EE from 2.36 to 2.96%, total phosphorus from 1.22 to 1.45%; and GE from 4504 to 4659 kcal/kg whereas, decreased the CF from 13.04 to 10.10%, thus enhancing the nutritional worth of WB.
- Fungal fermentation of DORB increased CP from 14.56 to 18.56%, EE from 1.13 to 1.36%, Ca from 0.30 to 0.47% and

GE from 3881 to 3986 kcal/kg whereas, decreased the CF from 12.85 to 7.38%, thus enhancing the nutritional worth of DORB.

- Optimum conditions for better growth of *Aspergilus niger* and economic fermentation on WB and/or DORB was found to be WB and water ratio of 70:30 (w/v), DORB and water ratio of 50:50 (w/v) for incubation period of 72 h at 37°C.
- The fermentation of wheat bran improved AMEn values of FWB (70:30), FWB (60:40) and FWB (50:50) by 21.67, 17.87 and 19.96% over raw counterpart, respectively. However, interaction effect for AMEn (nitrogen corrected) values of respective diets did not differ ($P>0.05$) significantly due to WB to moisture ratio.
- Fermentation of DORB after soaking it with different proportion of water viz. 70:30, 60:40 or 50:50 numerically improved its AMEn value by 5.04, 8.49 and 10.02%, respectively though. However, interaction effect for AMEn (nitrogen corrected) values of respective diets did not differ ($P>0.05$) significantly due to fermentation.
- Fermentation of the WB increased ($P<0.05$) *in vitro* pepsin-pancreatin digestibility of FWB (70:30), FWB (60:40) and FWB (50:50) by 9.09, 3.98 and 5.12% over their raw counterpart, respectively.
- Fermentation of the DORB increased ($P<0.01$) *in vitro* pepsin-pancreatin digestibility of FDORB (70:30), FDORB (60:40) and FDORB (50:50) by 7.54, 8.09 and 9.78% over the unprocessed DORB, respectively.
- There was significant ($P<0.01$) difference in ACHO content in raw or fermented WB. Fermentation of WB reduced ($P<0.01$) the ACHO content in FWB (70:30), FWB (60:40) and FWB (50:50) by 36.06, 24.06 and 24.29% over the raw WB, respectively.
- Fermentation of the DORB decreased ($P<0.01$) ACHO content in FDORB (70:30), FDORB (60:40) and FDORB (50:50) by

19.37, 9.61 and 32.87% over the unprocessed DORB, respectively.

- The available phosphorus (% total phosphorus) content of FWB (70:30), FWB (60:40) and FWB (50:50) was 62.35, 59.98 and 54.83%, respectively.
- The available phosphorus (% total phosphorus) contents of FDORB (50:50), FDORB (60:40) and FWB (70:30) were 45.88, 45.18 and 44.13%, respectively.
- FWB and FDORB when soaked with water at the ratio of 70:30 and 50:50 (w/v), the ADF content reduced by 13.01 and 10.88%, respectively over their raw counterparts.
- The BWG of the birds fed diets containing FWB at 5% inclusion level (1536 g) was relatively higher than control and other dietary treatment groups during 0-6 wks of age. Feeding of fermented of WB improved weight gain in broilers as compared to untreated WB, which was comparable to control diet containing corn-soya diet. The enzyme supplementation to the WB based diets was found beneficial in terms of body weight gain in broilers.
- When FWB inclusion levels were compared, feed intake showed a decreasing trend as the level of raw WB with or without enzyme increased in the diets. In general, feed intake decreased with the inclusion levels of WB in diet, either as raw or as fermented. In overall trend, feed intake was comparable at different inclusion levels of wheat bran either raw or fermented in broiler diets.
- Due to fermentation of WB, improvement in FCR in broilers was evident as compared to untreated WB, which was comparable to control diet containing corn-soya diet. The enzyme supplementation to the WB based diets was found beneficial in terms of FCR values in broilers.
- The protein and energy efficiency was comparable and was not affected by either fermentation or levels of inclusion of wheat bran with or without enzyme supplementation.

- The cost of feeding reduced significantly ($P<0.05$) in FWB over corresponding raw groups with or without enzyme supplementation. The addition of enzymes was also not beneficial in terms of economics of broiler production in raw wheat bran based diets. However, the feed cost per kg body weight gain or meat yield was lower in FWB fed groups.
- The N-retained (g/bird/d) and nitrogen retention (%) significantly ($P<0.01$) improved due to fermentation (FWB at 5% inclusion level) as compared to raw counterparts with or without enzymes supplementation. The dry matter metabolizabilty ($P<0.01$) and gross energy metabolizability differed significantly ($P<0.05$) due to the dietary treatments. When birds fed diets containing FWB have generally better ($P<0.05$) AMEn values than control group.
- The improvement in Ca and P-retention in present study was significant ($P<0.01$) in diets containing FWB over the raw counterparts and control.
- Slaughter traits of broilers revealed a non significant influence of treatments on blood loss, giblet yield and abdominal fat pad. However, feather loss, eviscerated yield, edible yield, and ready-to-cook (eviscerated with giblet weight) yield was significantly ($P<0.01$) different amongst various treatments. The weight of breast, back and neck did not differ significantly ($P>0.05$) amongst treatments. The weight of gizzard, heart and liver, bursa as per cent live weight showed a non significant difference due to different inclusion levels of raw WB, with or without enzymes and FWB. However, the birds offered fermented WB and WB with enzyme supplemented recorded significantly ($P<0.05$) higher weights of spleen than control and their raw counterparts.
- Due to fermentation, the serum biochemical parameters such as total cholesterol, triglycerides and uric acid were not affected due to dietary treatments. The cell-mediated immune response to phytohaemagglutinin-P was better in

all WB-fed groups. However, the humoral response to sheep red blood cells remained unaffected owing to the raw WB or its fermented form.

- FWB at 5 and 7.5% level reduced caeca microbes significantly ($P<0.01$) as compared to control and other dietary groups. Nevertheless, enzyme supplemented groups along with WB significantly ($P<0.01$) reduced caeca microbial count as compared to their respective non-enzyme supplemented groups (D_1, D_2 and D_3).
- Inclusion of raw WB, with or without feed enzyme and FWB in broiler diets did not bring out any change in the sensory quality attributes of meat.
- Fermentation of DORB improved weight gain in broilers as compared to untreated DORB, which was comparable to control diet containing corn-soya diet. The enzyme supplementation to the DORB based diets was/is not beneficial in terms of body weight gain in broilers.
- The feed intake and feed conversion ratio significantly improved in birds fed FDORB in comparison to their counterparts fed unprocessed DORB. The fermentation of DORB was beneficial in improving the growth performance. Moreover, supplementation of enzymes to unprocessed DORB-based diets or addition of enzymes with 5 and 7.5% of untreated DORB did not further improve the growth performance of broilers.
- The mortality occurred during feeding trail was well within the normal range and not found to be related to inclusion of fermented DORB at levels, investigated.
- Birds fed FDORB @ 5 and 7.5% inclusion level had significantly ($P<0.01$) better protein and energy efficiencies as compared to raw DORB with or without enzymes. Moreover, in birds fed on raw DORB with enzymes, the overall protein efficiency was statistically similar ($P<0.01$)

to control group. Similarly, energy conversion in birds fed raw DORB with enzymes showed similar trend. The birds received fermented DORB consumed significantly ($P<0.01$) lower energy per unit gain than in raw DORB with or without enzymes fed group.

- Feeding of fermented DORB decreased the cost of feed per kg gain or meat over raw DORB with or without enzymes fed groups, which was statistically comparable to control group. However, enzyme addition was not beneficial in reducing the feed cost of broiler production. It is inferred that, the feed cost per unit weight gain or meat yield apparently reduced due to fermentation.
- The N-retained (g/b) significantly ($P<0.05$) improved due to fermentation though the nitrogen retention and AMEn in group fed on FDORB was statistically non significant in comparison to untreated DORB, with or without enzymes fed group.
- Due to fermentation the DM metabolizabilty and gross energy metabolizability differed ($P<0.05$) due to the dietary treatments and the Ca and P retention improved due to fermentation.
- Slaughter traits, cut-up parts, vital organs and immune organs (% live weight) of broiler chickens revealed a non significant influence; remained statistically similar amongst various dietary treatments. Similarly, serum cholesterol also showed no significant difference due to different inclusion levels of raw DORB, with or without enzymes and FDORB than control birds. The birds fed on diets containing FDORB showed higher serum triglycerides as compared to their counterparts fed raw DORB and control. In uric acid concentration, no specific trend was observed due to fermentation.
- The birds fed diets containing FDORB and raw DORB with or without enzymes showed improved ($P<0.01$) immune

response measured in terms of cell-mediated immune response to phytohaemagglutinin-P compared to their counterparts fed raw or supplemented with enzymes. However, the immune response in terms of humoral response to SRBC did not show any significant ($P>0.05$) change in the dietary treatments.

- Birds fed FDORB @ 5 and 7.5% inclusion level reduced caeca microbes significantly ($P<0.05$) as compared to that of control and other dietary groups.
- The inclusion of raw DORB, with or without feed enzyme and FDORB in broiler diets did not bring out any change in the sensory quality attributes of meat.

CONCLUSION

- The *in vitro* study to ascertain the suitable moisture level for better growth of *Aspergilus niger* and economic fermentation indicated that substrate to moisture ratios (w/v) of 70:30 for wheat bran and 50:50 for de-oiled rice bran with an incubation period of 72 h at 37°C were found best for nutrient enrichment.
- Fungal fermentation of wheat bran increased CP from 14.89 to 19.78%, EE from 2.36 to 2.96%, total phosphorus from 1.22 to 1.45%; and GE from 4504 to 4659 kcal/kg whereas, decreased the CF from 13.04 to 10.10%, thus enhancing the nutritional worth of wheat bran.
- Fungal fermentation of de-oiled rice bran increased CP from 14.56 to 18.56%, EE from 1.13 to 1.36%, Ca from 0.30 to 0.47% and GE from 3881 to 3986 kcal/kg whereas, decreased the CF from 12.85 to 7.38%, thus enhancing the nutritional worth of de-oiled rice bran.
- Fermentation of wheat bran and de-oiled rice bran improved their AMEn, *In vitro* pepsin-pancreatin digestibility, available phosphorus (% total phosphorus) and reduced anti-nutritional factors such as phytates and acid detergent fiber.

- Dietary incorporation of fermented wheat bran up to 7.5% level beneficial in reducing feed cost of broiler production, nutrient utilization, immune competence and gut health but for growth performance.
- The feeding of diet containing 5% fermented de-oiled rice bran to broiler chicken showed beneficial results on growth performance, nutrient utilization, immune competence, gut health and feed cost of broiler production.

❖❖❖

Mini Abstract...

Mini Abstract

An attempt was made in the present study to enrich the nutritive value of high fibre wheat bran (WB) and de-oiled rice bran (DORB) through solid state fermentation technique utilizing *Aspergillus niger* and to evaluate and compare the feeding value of fermented wheat bran (FWB) and fermented de-oiled rice bran (FDORB) with or without enzyme supplementation in diet of broiler chicken on their growth performance, nutrient utilization, carcass traits, immune response, gut health and feed cost. Four separate experiments were carried out. The experiment 1 was made to ascertain the suitable moisture level for better growth of *Aspergilus niger* and economic fermentation for WB and DORB. In experiment 2, raw and the fermented WB and DORB were subjected to *in vitro* studies for evaluation of nutrient availability in terms of metabolizable energy (AMEn), dry matter and energy metabolizability, *in vitro* pepsin-pancreatin digestibility (IVPPD), available carbohydrates, acid detergent fiber and available phosphorus. In experiment 3 and 4, feeding trials were conducted to study the effect of fermentation of WB and DORB, with or without enzymes on the production performance and immunocompetence in broiler chickens, respectively. The overall results of the study indicated that the substrate to moisture ratios (w/v) of 70:30 for wheat bran and 50:50 for de-oiled rice bran with an incubation period of 72 h at 37°C were found best for nutrient enrichment. Fungal fermentation of WB increased CP from 14.89 to 19.78%, EE from 2.36 to 2.96%, total phosphorus from 1.22 to 1.45%; and GE from 4504 to 4659 kcal/kg whereas, decreased the CF from 13.04 to 10.10%, thus enhancing the nutritional worth of WB. Fungal fermentation of DORB increased CP from 14.56 to 18.56%, EE from 1.13 to 1.36%, Ca from 0.30 to 0.47% and GE from 3881 to 3986 kcal/kg whereas, decreased the CF from 12.85 to 7.38%, thus enhancing the nutritional worth of DORB. Fermentation of bran improved their AMEn, *In vitro* pepsin-pancreatin digestibility, available phosphorus (% total phosphorus) and reduced anti-nutritional factors such as phytates and acid detergent fiber. The study conducted to examine the effect of feeding fermented wheat bran with or without enzyme on growth and immune response in broilers envisaged that dietary incorporation of fermented wheat bran up to 7.5% level found beneficial for feed cost of broiler production, nutrient utilization, immune competence and gut health but for growth performance. The feeding of diet containing 5% fermented de-oiled rice bran to broiler chicken showed beneficial results on growth performance, nutrient utilization, immune competence, gut health and feed cost of broiler production.

Hindi Abstract...

लघु सारांश

वर्तमान अध्ययन में अधिक रेशे युक्त गेहूँ की भूसी (डब्लू. बी.) एवं तेल रहित चावल की भूसी (डी. ओ. आर. बी.) की पोषकता बढाने के लिए सॉलिड स्टेट किण्वन तकनीकी जिसमें एस्परजिलस नाइजर का उपयोग कर किण्वनित गेहूँ की भूसी (एफ. डब्लू. बी.) एवं किण्वनित तेल रहित चावल की भूसी (एफ. डी. ओ. आर. बी.) के साथ एन्जाइम पूरक एवं बिना एन्जाइम पूरक आहार का ब्रायलर चूजों के विकास निष्पादन, पोषक तत्वों का उपयोग, मांस के गुण, रोग प्रतिरोधी क्षमता, आँत स्वास्थ्य, एवं आहार मूल्य के तुलनात्मक आहारीय मूल्य का मूल्यांकन की कोशिक की गयी। इसकें लिए चार अलग–अलग प्रयोग किये गये। पहले प्रयोग में एस्परजिलस नाइजर के सर्वोत्मक विकास हेतु उपर्युक्त नमीं का स्तर तथा डब्लू. बी. एवं डी. ओ. आर. बी. के लिये आर्थिक किण्वन ज्ञात करने के लिये किया गया। दूसरे प्रयोग में कच्चे एवं किण्वनित डब्लू. बी. एवं डी. ओ. आर. बी. का प्रयोगशाला में (*इन वीट्रो*) अध्ययन किया गया जिसमें पोषक तत्वों की उपलब्धता जैसे उपापचय ऊर्जा, शुष्क पदार्थ एवं ऊर्जापचयता, *इनवीट्रो* पेपसीन. पैनक्रियाटीन पाचकता (आई. बी. पी. पी. डी.), उपलब्ध कार्बोहाइड्रेट, एसिड डिटर्जेन्ट रेशा एवं उपलब्ध फास्फोरस की उपलब्धता का मूल्यांकन किया गया। तीसरे एवं चौथे प्रयोग में आहारीय परीक्षण प्रयोग किए गये जिसमें फरमेन्टेशन का डब्लू. वी. एवं डी. ओ. आर. बी. के साथ एन्जाइम पूरक एवं बिना एन्जाइम पूरक आहार का प्रभाव ब्रायलर चूजों के विकास एवं रोग रोधी क्षमता का अध्ययन करने के लिए किया गया। इस अध्ययन के समग्र परिणामों से पता चलता है कि सबस्ट्रेट से सर्वोत्तम नमीं का अनुपात (डब्लू/वी) गेहूँ की भूसी के लिए 70:30 एवं तेल रहित चावल की भूसी के लिए 50:50 पायी गयी साथ में निवेशन का समय 37° सेन्टीग्रेड पर 72 घण्टे, जो पोषक तत्वों की उपलब्धता के लिये सर्वोत्तम पाया गया। डब्लू. बी. के फंगल किण्वन से प्रोटीन स्तर में (14.89 से 19.78 प्रतिशत), ई. ई. (2.36 से 2.96 प्रतिशत), कुल फास्फोरस में (1.22 से 1.45 प्रतिशत) तथा जी. ई. में (4504 से 4659 किलो कैलोरी/ किलो) तक बढोत्तरी पायी गयी। जबकि रेशे की मात्रा घटकर 13.04 से 10.10 प्रतिशत पायी गयी। इससे यह पता चलता है डब्लू. बी. की पोषकता में वृद्धि हुयी। डी. ओ. आर. बी. के फंगल किण्वन से प्रोटीन में (14.56 से 18.56 प्रतिशत), ई. ई. (1.13 से 1.36 प्रतिशत) , कैल्शियम (0.30 से 0.47 प्रतिशत) तथा जी. ई. (3881 से 3986 किलो कैलोरी /किलो) तक की बढोत्तरी पायी गयी। जबकि रेशे की मात्रा 12.85 से घट कर 7.38 प्रतिशत पायी गयी। इससे यह पता चलता है कि डी. ओ. आर. बी. की पोषकता में वृद्धि हुयी। भूसी के किण्वन से ए. एम. इ. एन. *इन वीट्रो* पेपसीन– पेनकियाटीन पाचकता, उपलब्ध फास्फोरस (प्रतिशत कुल फास्फोरस) मे वृद्धि पायी गयी। तथा पोषक रोधी घटकों जैसे– फाइटेट एवं एसिड डिटर्जेन्ट रेशे में कमी पायी गयी। किण्वनित गेहूँ की भूसी के साथ इन्जाइम एवं बिना इन्जाइम पूरक युक्त आहार का ब्रायलर चूजों के विकास एवं रोधी क्षमता का प्रभाव यह दर्शाता है कि किण्वनित गेहूँ की भूसी को आहार में 7.5 प्रतिशत स्तर तक मिलाने पर ब्रायलर उत्पादन में ब्रायलर के आहार मूल्य को कम करने, पोषक तत्वों का उपयोग, रोग रोधी क्षमता, तथा आँत स्वास्थ्य पर लाभप्रद प्रभाव पाया गया। जबकि आहार में 5 प्रतिशत तक किण्वनित तेल रहित चावल की भूसी को मिलाने पर ब्रायलर के विकास निष्पादन, पोषक तत्वों का उपयोग, रोगरोधी क्षमता, आँत स्वास्थ्य तथा आहार मूल्य को कम करने हेतु ब्रायलर उत्पादन में लाभप्रद प्रभाव पाया गया।

References...

References

Abalaka, M.E and Yahaya, D. (2010) Assessment of the performance of chicks fed with cereal wastes enriched with single cell protein – *Candida tropicalis*. *AU. J. Technol.,* **13(4):** 261-264

Abasiekong, S.F. (1991). Effect of dietary inclusion of re-fermented sorghum residue on broiler chicken performance. *Arch. Anim. Nutr. (Berlin),* **41 (2):** 187-194.

Abaza, I.M, Ali, M.N. and Hassan, M.S. (2004). Nutritional and physiological studies on improving the utilization of wheat bran in laying hen diets. *Egypt. Poult. Sci.*, **24:** 1015-1031.

Abbas, K.K., Lichtman, A.H. and Prober, J.S. (1991). Cellular and molecular immunology. W.B. Saunders Co., Philadelphia, P.A.

Adams, O. L. and Naber, E. C. (1969). Effect of physical and chemical treatments of grains on growth and feed utilization by chicks. 1. The effect of water and acid treatments of corn, wheat, barley and expanded or germinated grains on chick performance. *Poult. Sci.*, **48:** 853-858.

Adrizal, Palo, P. E., and Sell, J. L. (1996). Utilization of defatted rice bran by broiler chickens. *Poultry Sci.*, **75 :** 1012-1017.

Aduku, A.O. (1993). Tropical feedstuff analysis table. Department of Animal Science. ABU, Zaria-Nigeria.

Ahmed, A. S., Amer, H. A. and Ibrahim, I. M. (1989). Influence of dietary mineral imbalance on the incidence of urolithiasis in Egyptian calves. *Archiv fur-Experimentelle-Veterinarmedizin.,* **43:** 73-77.

Akeson, W.R. and Stahman, M.A. (1964). A pepsin pancreatin digest index of protein quality evaluation. *J. Nutr.,* **83**: 257-261.

Akhtar, N., Asghar, M. and Mahmood, Z. (1997). Bioconversion of agro-industrial wastes to protein biomass/isolates by *Arachniotus sp.* and evaluation its protein quality by limiting amino acid estimation. *J Appl. Poult. Sci.*, **17(1-2)**: 52-54.

Ali, M.N., Hassan, M.S. and Abaza, I.M. (2006a). Effect of improving the utilization of wheat bran on productive and physiological performance for local laying hens. *Egypt. Poult. Sci.*, **26:** 137-158.

Ali, M.N., Namra, M.M.M. and Wahed, A. and Hala, M. (2006b). Improving the utilization of wheat bran in quail. *Egypt. Poult. Sci*., **26:** 557-577.

Alka-Sharma, X. and Kapoor, A. (1996). Levels of anti-nutritional factors in pearl millet as affected by processing treatment and various types of fermentation. *Plant Foods Human Nutr.*, **49**: 241- 252.

Allen, C. M., Bedford, M. R. and McCracken, K. J. (1997). Effect of level of wheat inclusion and enzyme supplementation on diet metabolizability and broiler performance. *Br. Poult. Sci*., **38**: 525-526.

Almquist, D. (1948). Proteins and amino acids in nutrition, edited by Sahyun, p. 231.

Annison G., Moughan, P. J. and Thomas, D. V. (1996). Nutritive activity of soluble rice bran arabinoxylans in broiler diets. *British Poult.Sci.*, **36 (3):** 479 – 488

Annison, G. (1990). Polysaccharides composition of Australian wheats and the digestibility of their starches in broiler chicken diets. *Aust., J. Expt. Agric.*, **30**: 183-186.

Annison, G. (1991). Relationship between the levels of non-starch polysaccharides and the apparent metabolizable energy of wheat assayed in broiler chickens. *J. Agric. Food Chem.*, **39**: 1252-1256.

Annison, G. and Choct, M. (1991). Antinutritive activities of cereal non-starch polysaccharides in broiler diets and strategies for minimizing their effects. *World's Poult. Sci. J.*, **47**: 232–242.

Annison, G. and Choct, M. (1993). Enzymes in poultry diets. In: Wenk, C. and M. Boessinger, ed., Enzymes in Animal Nutrition. Kartaus Ittingen. Thurgau, Switzerland, pp. 61-68.

Anupama; Ravindra, P. (2000), Value-added food: single cell protein. *Biotechnol. Adv.*, **18**: 459-479.

Anupama; Ravindra, P. (2001), Studies on production of single cell protein by *Aspergillus niger* in solid state fermentation of rice bran. *Brazilian Arch. Biol. Technol.*, **44** (1), 79 – 88.

AOAC (1990). Officials Methods of Analysis (15th edn.) Association of official analytical chemists. Washington, D.C.

AOAC. (2000). Official Methods of Analysis. Association of Official Analytical Chemists. 17th Ed., Gaithersburg, Maryland, USA.

Attia, Y. A. and Rahman, S. A. (1996). Effect of enzyme supplementation on performance, carcass characteristics and digesta passage time of broiler chicks fed different cereal grains. *Egypt. J. Anim. Prod.*, **33**: 125-144.

Bailey, J. S., Blakenship, L. C. and Cox, N. A. (1991). Effect of fructo-oligosaccharide on *Salmonella* colonization of the chicken intestine. *Poult. Sci.*, **70:** 2433-2438.

Bajwa, M. A., Aziz, T. and Hasmi, A. S. (1991). Production of fungal biomass protein from alkali treated rice straw by *Arachniotus sp. J. Appl. Poult. Sci.,* **1(2):** 79-82.

Bakshi, I. S. (1971). Studies on the nutritive value of de-oiled rice polish for poultry. M. V. Sc. (Poultry Science) thesis, JNKVV, Jabalpur. Cited: Sathe, B. S. *Poult. Guide*, **VIII (12)**: 12-15.

Bakshi, Y., Creger, C.K. and Couch, J.R. (1964). Studies on guar meal. *Poult. Sci.*, **43:** 1302.

Barber, S., Benedito-de, B. C., Flores, M. J. and Montes, J. J. (1978). Toxic constituents of rice bran. I. trypsin inhibitor activity of raw and heat treated rice bran. *Rev. Agroquim. Aliment.*, **18:** 80-88.

Barrier-Guillot, G.C., Casado, P., Maupetit, P., Jandreville, C. and Gatel, F. (1996). Wheat phosphorous availability. 2. *In vivo* study in broilers and pigs; relationship with endogenous phytasic activity and phytic phosphorous content in wheat. *J. Sci. Food Agric.*, **70**: 69-74.

Bartinik, M. and Szafranska, I. (1987). Changes phytate content and phytase activity during germination of some cereals. *J. Cereal Sci.*, **5:** 23-28.

Bashir, S., Hashmi, A.S. and Gilani, A. H. (1990). Bioconversion of wheat bran to protein concentrate with Candida utilis. M.Sc. Thesis, Department of Animal Nutrition, University of Agriculture, Faisalabad, Pakistan.

Bedford, M.R. (1995). Mechanism of action and potential environmental benefits from the use of feed enzymes. *Anim. Feed Sci. Technol.*, **53:** 145–155.

Bedford, M.R. (1996). The effect of enzymes of digestion. *J. Appl. Poult. Res.*, **5:** 370-378.

Bedford, M.R., Classen, H.L. and Campbell, G.L. (1991). The effect of pelleting, salt, and pentosanase on the viscosity of intestinal contents and the performance of broiler fed rye. *Poult. Sci.*, **70**:1571-1577

Benazir J.F., Suganthi, R., Hari, A., Kumar, R., Aswathi, V., Niraimathi, M.P., Mala, G., Sukanya, M.K., Santhi, R. (2011). Bio utilization of agroindustrial waste in solid state fermentationby *Aspergillus niger* for the production of protease. *Asiatic J. Biotechnol. Resources*, **2(04)**: 422-435.

Bengtsson, S., Anderson, R., Westerlund, E. and Aman, P. (1992). Content, structure and viscosity of soluble arabinoxylans in rye grain from several countries. *J. Sci. Food Agric.*, **58:** 331–337.

Bergman, E. L., Autio, K. and Sandberg, A. S. (2000). Optimal conditions for phytate degradation, estimation of phytase activity, and localization of phytate in barley. *J. Agri. Food Chem.*, **48**: 4647- 4655.

Bhanja, S.K. (1992). The nutritive value of de-oiled rice bran for the Japanese quails. M.V. Sc Thesis submitted to Deemed University, IVRI, Izatnagar, India.

Bhar, R. and Mandal, A.B. (2004). Feed resources for pigs, feedstuffs for livestock and poultry, edited by Mandal, A.B., Elangovan, A. V., Tyagi Praveen Kumar, Tyagi Pramod Kumar, Singh Rajvir, Pub by lead centre, NATP, CARI, Izatnagar 170-178.

Bhatia , I. S. (1969). Evaluation of feed stuffs available in India for protein quality and energy value. Final report (1964-69), PAU, Ludhiana.

Bhatti, B. M., Talat, T. and Sardar, R. (2001). Estimation of aflatoxin B1 in feed ingredient and compound poultry feeds. *Pakistan Vet. J.*, **21 (2)**: 57-60.

Bhavsar, K., Shah, P. Soni, S. K. and Khire, J. M. (2008). Influence of pretreatment of agriculture residues on phytase production by *Aspergillus niger* NCIM 563 under submerged fermentation conditions. *African J. Biotechnol.,* **7(8)**:1101-1106.

Bhutia, S. (2006). Nutritive value of toasted guar meal with or without enzyme supplementation in broiler quail ration. M.V.Sc thesis submitted to Deemed University, I.V.R.I., Izatnagar.

Bonnin, E., Saulnier, L., Branel, M., Marot, C., Lesage-Meessen, L., Asther, M. and Thibault, J.F. (2002). Release of ferulic acid from agroindustrial by-products by the cell wall-degrading enzymes produced by *Aspergillus niger* I-1472. *Enzyme Microb. Technol.,* **31**: 1000-1005.

Bootwalla, S. (2005). Poultry and population on the Asian subcontinent. *World's Poult.,* **21**:10-12.

Brahma, T. C. and Siddiqui, S. M. (1979). A preliminary study on the utilization of toasted guar meal in broiler rations. *Indian Poult. Gaz.,* **62:** 133-38.

Brahma, T. C., Siddiqui, S. M. and Reddy, S. V. (1979). A study as the utilization of toasted guar meal and without supplemental amino acids in broiler rations. *Indian Poult. Sci.,* **14:** 41.

Brillouet, J.M., Joseleau, J.P., Utille, J.P. and Lelievre, D. (1982). Isolation, purification and characterization of complex heteroxylans from industrial wheat bran. *J. Agric. Food Chem.*, **30:** 488-495.

Butt M. S., Qamar, I., Anjum, M., Abdul Aziz, F., and Randhawa, A.M. (1998). Development Of Minerals Enriched Brown Flour By Utilizing Wheat Milling By-Products. *Internet J. Food Safety*, **3:**15-20

Cabrera, M.R. (1994). Effect of sorghum genotypes and particle size on milling characteristics and performance of finishing pigs, broiler chicks and laying hens. *M.V.Sc. thesis, Kansas state University, Manhattan*.

Carpenter, K.J. (1973). Damage to lysine in food processing: its measurements and its significance. *Nutr. Abstr. Rev.,* **43**: 423-451.

Cavalcanti, B.W. and Behnke, C.K. (2004). Effect of wheat bran phytase subjected to different conditioning temperatures on phosphorus utilization by broiler chicks based on body weight and toe ash measurements. *Int. J. Poult. Sci.*, **3**: 215-219.

Chang, C.W. (1967). Study of phytase and fluoride effects in germinating corn seeds. *Cereal Chem.*, **44**: 129-142.

Chavan, J. K. and Kadam, S. S. (1989). Nutritional improvement of cereals by fermentation. *CRC Cr. Rev. Food Sci. Technol.*, **28(5)**: 349-352.

Chavan, U.D., Chavan, J.K. and Kadam, S.S. (1988). Effect of fermentation on soluble protein and *in vitro* protein digestibility of sorghum, green gram and sorghum green blends. *J. Food Sci.*, **53:** 1574-1578.

Chee, K. M., Chun, K. S., Huh, B. D., Choi, J. H., Chung, M. K., Lee, H. S., Shin, I. S. And Whang, K. Y. (2005). Comparative Feeding Values of Soybean Hulls and Wheat Bran for Growing and Finishing Swine. *Asian-Aust. J. Anim. Sci.*, **18 (6)**: 861- 867

Cheeke, P. R. (1991). Applied Animal Nutrition. Feeds and Feeding, New York.

Chisti, Y. (1999). Solid substrate fermentations, enzyme production, food enrichment. In Encyclopedia of Bioprocess Technology: Fermentation, Biocatalysis, and Bioseparation, Vol 5, ed. By Flickinger MC and Drew SW. Wiley, New York, pp. 2446– 2462.

Choct, M. and Annison, G. (1990). Anti nutritive activity of wheat pentosans in poultry diets. *Br. Poult. Sci.*, **31**: 809-819.

Choct, M. and Annison, G. (1992a). Antinutritive effect of wheat pentosans in broiler chickens. Role of viscosity and gut microflora. *Br. Poul. Sci.*, **33:** 821- 834.

Choct, M. and Annison, G. (1992b). The inhibition of nutrient digestion by wheat pentosans. *Br. J. Nutr.*, **67**: 123-132.

Choct, M., Hughes, R. J., Wang, J., Bedford, M. R., Morgan, A. J. and Annison, G. (1996). Increased antinutritive activity of non-starch polysaccharides in chickens. *Br. Poult. Sci.*, **37**: 609-621.

Choct, M., Hughes, R.J. and Bedford, M.R. (1999). Effect of xylanase and individual bird variation, starch digestion through out the intestine, and ileal and caecal volatile fatty acid production in chickens fed wheat. *Br. Poult. Sci.*, **40**: 419-422.

Choct, M., Hughes, R.J., Trimble, R.P., Angkanaporn, K. and Annison, G. (1995). Non-starch polysaccharide-degrading enzymes as increase the performance of broiler chickens fed wheat of low apparent metabolizable energy. *J. Nutr.,* **125:** 485-492.

Chompreeda, P. T. and Fields, M. L. (1981). Effects of heat and fermentation on the extractability of minerals from soybean meal and corn meal blends. *J. Food Sci.,* **49:** 566-570.

Christopher, G.I., Offiong S., and Idiong, I.C. (2007). Effect of the replacement of maize with wheat offal in broiler finisher diets ongrowth performance and feed cost. *J. Central Eur. Agric.*, **8(1)**: 33-38.

Chutmanop, J., Chuichulcherm, S., Chisti, Y. and Srinophakun, P. (2005). Protease production by Aspergillus oryzae in solidstate fermentation using agroindustrial substrates. *J.Chem. Technol. Biotechnol.*, **83:**1012–1018.

Cilliers, S. C., Sales, J., Hayes, J. P., Chwalibog, A. and Preez, J. J. D. (1999). Comparison of metabolisable energy values of different foodstuffs determined in ostriches and poultry. *British Poultry Sci.* **40(4)**: 491-494.

Classen, H. L . (1996). Cereal grain starch and exogenous enzymes in poultry diets. *Anim. Feed Sci. Technol.,* **62:** 21-27.

Clegg, K. M. (1956). The application of the anthrone reagent to the estimation of starch in cereals. *J. Sci. Food Agri.*, **7**: 40-44.

Cook, M. E. (1991). Nutrition and the immune response of the domestic fowl. *Cr. Rev. Poult. Biol.,* **3**: 167-190.

Corrier, D. E. and DeLoach, J. R. (1990). Evaluation of cell-mediated, cutaneous basophil hypersensitivity in young chickens by an interdigital skin test. *Poult. Sci.* **69:** 403-408.

Couch, J.R., Bakshi, Y.K., Ferguson, T.M., Smith, E.B. and Creger, C.R. (1967). The effect of processing on the nutritional value of guar meal for broiler chicks. *Br. Poult. Sci.,* **8:** 243-50.

Courtin, C.M., Swennen, K., Broekaert, W.F., Swennen, Q., Buyse, J., Decuypere, E., Michiels, CW., Ketelaere, B.D. and Delcour, J.A. (2008). Effects of dietary inclusion of xylooligosaccharides, arabinoxylooligoscaccharides and soluble arabinoxylan on the microbial composition of caecal contents of chickens. *J. Sci. Food Agric.*, **88(14)**: 2517-2522.

Couto, S. R. and Sanroman, M. A. (2006). Application of solid-state fermentation to food industry - A review. *J. Food Engineering,* **76**: 291–302.

Dale, N. (1996). The metabolizable energy of wheat by-product. *J.Appl. Poul. Sci.,* **5**:105-108.

Darwazeh, M. M. (2010). Effects of Rumen Filterate Fermented Wheat Bran on Performance of Finishing Broiler Chicken. Thesis is submitted to An-Najah National University, Nablus, Palestine.

Daubresse, P., Ntibashirwa, S., Gheysen, A. and Meyer, J.A. (1987). A process for protein enrichment of cassava by solid substrate fermentation in rural conditions. *Biotechnol. Bioengineering,* **29**: 962–968.

Deolankar, R. P. and Singh, K. S. (1979). Trypsin inhibitor, mineral availability, and performance of broiler chickens fed on diets based on rice bran. *Anim. Feed. Sci. Tech.,* **4**: 133-141.

Di Lena, G., Patroni, E. and Quaglia, G. B. (1997). Improving the nutritional value of wheat bran by a white-rot fungus. International *J.Food Sci. Technol.,* **32**: 513-519

Dinani, O. P. (2009). Solid substrate fermentation of toasted guar meal and its comparision with or without enzyme supplementation in broiler quail ration. M.V. Sc Thesis submitted to Deemed University, IVRI, Izatnagar, India.

Dinani, O. P., Tyagi Pramod Kr., Shrivastav, A. K. and Tyagi, P. K. (2010). Effect of feeding guar meal vis-à-vis toasted guar meal with or without enzyme supplementation on performance broiler quail. *Indian J. Poult. Sci.,* **45(2)**: 150-156.

Dirar, H.A. (1992). Traditional fermentation technology and food policy in Africa. *Appropriate Technol.,* **193**: 21-23.

Dixit, R. and Baghel, R. P. S. (1998). Effect of feeding sorghum instead of maize along with groundnut cake and fish meal on carcass traits of broilers. *Indian J. Anim. Prod. Managt.,* **14**: 134-136.

Domacinovic, M., Sencic, D. and Antunovic, Z. (1999). Micronized cereals in chicken fattening. *Czech J. Anim. Sci.,* **44 (5)**: 225-228.

Douglas, J.H., Sullivan, T.W., Abdul Kadir, R. and Rupnow, J.H. (1991). Influence of infra-red (micronization) treatment on the nutritional value of corn and low- and high- tannin sorghum. *Poult. Sci.,* **34 (3)**: 389-392.

Duncan, D. B. (1955). Multiple range and multiple-F tests. *Biometrics,* **11**: 1-42.

Durand, A. and Chereau, D. (1988). A new pilot reactor for solid-state fermentation: Application to the protein enrichment of sugar beet pulp. *Biotechnol Bioeng.,* **31(5):** 476-486.

Dusel, G., Kluge, H., Jeroch, H. and Simon, O. (1998): Xylanase supplementation of wheat based rations for broilers: Influence of wheat characteristics. *J. Appl. Poultry Res.,* **7**: 119-131

Eeckhaut, V., Van Immerseel, F., Dewulf, J., Pasmans, F., Haesebrouck, F., Ducatelle, R., Courtin, C. M. , Delcour, J. A. and Broekaert, W. F. (2008). Arabinoxylooligosaccharides from wheat bran inhibit *Salmonella* colonization in broiler chickens. *Poult. Sci.,* **87**: 2329-2334.

Eeckhout, W. and de Paepe, M. (1994). Total phosphorus, phytate-phosphorus and phytase activity in plant feedstuffs. *Anim. Feed Sci. Technol.,* **47**: 19–29.

Eeckhout, W. and De Paepe, M.M. (1993). Total phosphorus and phytase activity in plant feedstuffs. *Animal Feed Sci. Feed Technol.,* **47**: 19-29.

El Hag, M.E., Abdullahi, H.E. and Nabila, E.Y. (2002). Effect of fermentation and dehulling on starch, total polyphenols, phytic acid content and *in vitro* protein digestibility of pearl millet. *Food Chem.,* **77**: 193-196.

Elangovan, A. V., Deo, Chandra, Mandal, A. B., Singh, D. P., Shrivastava, H. P. and Johri, T. S. (2002). Effect of enzymes supplementation in diets with varing energy levels on egg production of CARI-Red layers. *Proc. X Int. Cong. Asian-Austr. Assoc. Anim. Prod. Soc.* (AAAP), Ashoka Hotel, New Delhi, Sept. 23-27, PS II.59, p.235.

El-Tinay, A. H., Abdel-Gadir, A. M. and El-Hidai, M. (1979). Sorghum fermented kisra bread. I. Nutritive value of kisra. *J. Sci. Food Agric.*, **30**: 859-862.

Eshwaraiah, R., Rao, P. V. and Reddy, C. V. (1986). Evaluation of chemical and nutrient composition in raw, de-oiled and parboiled rice polishing and maize. *Indian J. Anim. Sci.*, **21**: 72-74.

Eshwaraiah, R., Rao, P. V. and Reddy, C. V. (1988). Effect of autoclaving and solid substrate fermentation of raw, de-oiled and parboiled rice polishing in broiler diets. *Indian J. Anim Sci.*, **58**: 377-381.

Evans, C. S. (1985). Properties of b-glucosidase from the wood rotting fungus, *Cariolus veskolor. App. Microbiol Biotech.*, **22**: 128-131.

Farrell, D. J. and Martin, E. A. (1998). Strategies to improve the nutritive value of rice bran in poultry diets. I. The addition of food enzymes to target the non-starch polysaccharide fractions in diets of chickens and ducks gave no response. *British Poult. Sci.*, **39 (4)**: 549-554.

Farrell, D. J. Martin, E, du Preez, J.J., Bongarts, M., Betts, Sudaman, A. and Thomson, E. (1993). The beneficial effect of a microbial feed phytase in diets of broiler chicken and ducklings. *J. Anim. Physiol. Anim Nutr.*, **69**: 278-283.

Farrell, D. J., (1994). Utilization of rice bran in diets for domestic fowl and ducklings. *World's Poult. Sci. J.*, **50**:115-131.

Fengler, A. I., Pawlik, J. R. and Marquardt, R. R. (1988). Improvement in nutrient retention and changes in excreta viscosities in chicks fed rye-containing diet supplemented with fungal enzymes, sodium taurocholate and penicillin. *Canadian J. Anim. Sci.*, **68**: 483-491.

Fossati, P. and Lorenz, P. (1982). Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. *Clin. Chem.*, **28**: 2077-2080.

Friesen, O. D., Guenter, W., Marquardt, R. R. and Rottor, B.A. (1992). The effect of enzyme supplementation on the apparent metabolizable energy and nutrient digestibilities of wheat, barley, oats and rye for the young broiler chick. *Poult. Sci.,* **71**: 1710-1721.

Fry, R. E., Allred, J. B., Jensen, L. S. and McGinnis, J. (1958). Influence of enzyme supplementation and water treatment on the nutritional value of different grains for poults. *Poult. Sci.,* **37**: 372-375.

GAFTA (1995).The Grain and Feed Trade Association,USA. Method **27:** pp.108-109

Gallinger, C. I., Suarez, D. M. and Irazustam, A. (2004). Effects of rice bran inclusion on performance and bone mineralization in broiler chicks. *J. Appl. Poult. Res.,* **13:** 183-190

Ghildyal, N. P., Ramakrishna, S. V. and Nirmala Devi. P. (1981). Large scale production of a proteolytic enzyme by solid state fermentation. *J. Food Sci. Technol.,* **18**: 248- 251

Godoy, S., Chicco, C., Meschy F. and Requena, F. (2005). Phytic phosphorus and phytase activity of animal Feed Ingredients. INCI v.30 n.1 Caracas ene. 2005.

Gohl, B. and Thombe, S. (1976). Digestibility coefficients and metabolizable energy of barley diets for layers as influenced by geographical area of production. *Poult. Sci.*, **55**: 2169-2174.

Gopalkrishnan, M.V. and Prakash, J. (2000). Optimum time requirement for enzymatic hydrolysis of food proteins. *J. Food Sci. Tech.,* **37**: 319-322.

Gous, R.M., Kuyper, M.A. and Dennison, C. (1982). The relationship between tannic acid content and metabolizable energy concentration of some sorghum cultivars. *South African J. Anim. Sci.,* **12**: 39–44.

Guillard, R.R.L. (1973). Division rates. In Stein, J.R. ed. Handbook of physiological methods: culture methods and growth measurements. Cambridge University Press, Cambridge. pp.108-132.

Gujral, D. (2005). Efficacy of certain oligosaccharides as substitute for dietary antibiotic for optimum performance of meat type quails: Ph.D. Thesis, Deemed University, IVRI, Izatnagar.

Gunashree, B.S., Venkateswaran, G. and Jagannatharao, R. (2007). Efficacy of Aspergillus niger phytase on the availability of phytate phosphorus. *Indian J. Poult. Sci.*, **42 (3)**: 251-256.

Gunawan, B. and Tangendjaja, B. (1988). The effect of long storage of rice bran for growing broiler chickens. *Ilmu dan Peternakan*., **3**:135-138.

Gupta, J. J., Yadav, B.P.S. and Gupta, H.K. (1995). Nutritional value of jack bean for broiler. *Indian J. Poult. Sci.*, **30 (2)**: 112-116.

Hamad, A. M. and Fields, M. L. (1979). Evaluation of the protein quality and available lysine of germinated and fermented cereal. *J. Food Sci.*, **44**: 456-463.

Hamilton, R.M.G. and Kennie, J. (1997). The effects of lighting program, ingredient particle size and feed form on the performance of broiler turkeys. *Canadian J. Anim. Sci.*, **77 (3)**: 503-508.

Hamilton, R.M.G. and Proudfoot, F.G. (1995). Ingredient particle size and feed texture : effects on the performance of broiler chickens. *Anim. Feed Sci. Technol.*, **51(3-4)**: 203-210.

Hammond, N. A. (1994). Method for stabilizing rice bran and rice bran products. Bran Technol. Inc., Baton Touge, LA, USA. p. 9.

Han, Y.W. and Wilfred, A.G. (1988). Phytate hydrolysis in soybean and cottonseed meals by Aspergillus ficuum phytase. *J. Agric. Food Chem.*, **36**: 259-262.

Haugh, W. and Lantzsch, H. J. (1983). Sensitive method for the rapid determination of phytate in cereals and cereal products. *J. Sci. Food. Agri.*, **34**: 1423-1426.

Hegde, S., Kavitha, S., Varadaraj, Varadaraj, M. C. and Muralikrishna, G. (2006). Degradation of cereal bran polysaccharides-phenolic acid complexes by *Aspergillus niger* CFR 1105. *Food Chem.*, **96**: 14-19.

Henry, R. J. (1985). A comparison of non-starch carbohydrates in cereal grains. *J. Food Sci. Agric.*, **36**: 1243-1253.

Hill, F.W. and Anderson, D.L. (1958). Comparison of metabolizable energy and productive energy determination with the chicks. *J. Nutr.*, **64**: 587–603.

Hill, F.W. and Renner, R.C. (1963). Effect of heat treatment on the metabolizable energy value of soybeans and extracted soybeans flakes for the hen. *J. Nutr.,* **80**: 375-388.

Houston, D. F. (1972). Rice bran and polish. In: *"Rice Chemistry and Technology"*. Ed. Houston D. F., p. 272. American Association of Cereal Chemistry, Inc., St. Paul, MN.

Hussar, N. and Robblle, A. R. (1962). Effects of pelleting on the utilization of feed by the growing chicken. *Poult. Sci.*, **41**: 1489-1493.

Icchponani, J. S. and Makkar, C. S. (1989). Feeding of de-oiled rice bran to poultry. A false economy. *Poult. Guide,* **XXVI (2)**: 27-33.

ICMSF (1978). Sampling for microbial analysis- Principle and specific application in microorganism in food. University of Toranto Press, 67-75.

Igbasan, K.C. and Guenter, W. (1996). The enhancement of nutritive value of peas for broiler chickens: an evaluation of micronization and dehulling process. *Poult. Sci.*, **75(10)**: 1243-1252.

Ikegami, S., Tsuchikaski, F., Harada, H., Tsuchikaski, N., Nishidi, E. and Innami, S. (1990). Effect of viscous indigestible polysaccharides on pancreatic-biliary secretion and digestive organs in rats. *J. Nutr.*, **120**: 353-360.

Ingram, G.R., Ricson, W.W., Cravens, W.W. and Elvehjem, C.A. (1949). Evaluating soybean oil meal protein for chick growth by enzymatic release of amino acids. *Poult. Sci.*, **28**: 898-902.

Ishihara, N., Chu, D. C., Akachi, S. and Juneja, L. R. (2002). Preventive effect of partially hydrolyzed guar gum on infection of *Salmonella enteriditis* in young and laying hens. *Poult. Sci.*, **79:** 689-697.

Jacob, Z. (1991). Enrichment of Wheat Bran by *Rhodotorula gracilis* through Solid-State Fermentation. *Folia Microbiol.*, **36 (1)**: 86-91.

Janssen, W.M.M.A. and Carre, B. (1985). Influence of fibre on digestibility of poultry feeds, in: Haresign, W. and Cole, D.J.A. (Eds.) *Recent Advances in Animal Nutrition, pp. 71—86, Butterworths, London.*

Juliano, B. O. (1985). Rice: Chemistry and Technology. The American Association of cereals chemists, Inc., St. Poul., Minnesota, USA.

Kang, S. W., Park, Y. S., Lee, J. S., Hong, S. I. and Kim, S. W. (2004). Production of cellulases and hemicellulases by *Aspergillus niger* KK2 from lignocellulosic biomass, *Biores. Technol.*, **91**: 153-156.

Kavitha, S., Shyamala, H., Muralikrishna, G., Varadaraj, M.C. and Rati Rao, E. (2004). Starch and cell wall degrading enzymes from fungal organism grown on cereal and millet brans. Eur. Food Res. Technology.

Ke, L., Wu, Q. and Zhang, D. (2011). Bioconversion of rape straw into a nutritionally enriched substrate by Ganoderma lucidum and yeast. *African J. Biotechnol.,* **29**: 5648-5653.

Kettunen, H., Tiihonen K., Peuranen S., Saarinene M.T., and Remus, J.C. (2001). Dietary betaine accumulates in the liver and intestinal tissue and stabilizes the intestinal epithelial structure in healthy and coccidian-infected broiler chicks. *Comp. Biochem. Physiol. A Mol.Integr. Physiol.*, **130**: 759-769.

Khan S. H., Hashmi, A. S., Khan, M. L. and Rajoka, M. I. (1990). Bioconversion of de-fatted rice polishing to protein concentrate and its biological evaluation in broiler chicks. Proc. 3rd Int. Cong., Pak. Vet. Med. Assoc., November 28-29, Islamabad, pp. 331-339.

Kiani, A. H.I. (1989). Bioconversion of de-fatted rice polishing with Candida utilis to protein concentrate. M.Sc. Thesis, Department of Animal Nutrition, University of Agriculture. Faisalabad, Pakistan.

Konietzny, U., Jany, K-D. and Greiner, R. (2006). Phytate - an undesirable constituent of plant-based foods? *J. für Ernährungsmedizin*, **8 (3)**: 18-28.

Latshaw, J.D. (1974). Soybean processing and its effect on the laying hen. *Poult. Sci.*, **53(4)**: 1342-1347.

Laufenberg, G., Kunz, B. and Nystroem, M. (2003), Transformation of vegetable waste into value added products: (A) the upgrading concept; (B) practical implementations. *Bioresource Technol.*, **87(2)**: 167-198.

Lee, J. T., Connor, Appleton, S., Bailey, C. A. and Cartwright, A. I. (2005). Effects of gaur meal by products with and without Mannanase (Hemicell) on broiler performance. *Poult. Sci.,* **84:** 1261-67.

Lee, J. T., Bailey, C. A. and Cartwright, A. L. (2003a). Mannanase ameliorates viscosity associated depression of growth in broiler chickens fed guar germ and hull fractions. *Poult. Sci.,* **82:** 1925-31.

Lee, J.T., Bailey, C.A. and Cartwright, A.L. (2003b). Guar meal germ and hull fractions differently affect growth performance and intestinal viscosity of broiler chickens. *Poult. Sci.,* **82:** 1589-95.

Leeson, S. and Summer, J.D. (2001). Scott's Nutrition of the Chicken. 4th ed. University Books, Guelph.

Leeson, S. and Summers, J.D. (2008). Commercial Poultry Nutrition. 4th ed. Publ. Univ. Books, Guelph, Ontario, Canada.

Lodhi, G. N., Singh, D. and Ichhponani, J. S. (1976). Varition in nutrient content of feed stuffs rich in protein and reassessment of chemical method for metabolisable energy estimation for poultry. *J. Agric. Sci.,* **86:** 293-304.

Lynch, P. B. and Caffrey, P. J. (1997). Phosphorus requirements for animal production. *In Phosphorus Loss from Soil Water, H. Tunney, O. T. Carton, P. C. Brookes, and A. E. Johnston, (Eds.) CAB Int., Wallingford, UK.*

Mahmood, Z., Hasmi, A. S. and Ashraf, A. (1991). Isolation of Mycotoxins produced by an antagonistic fungal organism, *Archnitous sp. Pak. J. Agri. Sci.,* **27(4)**: 314-316.

Mandal A. B. and Pathak, N. N. (1996). Comparative evaluation of metabolizable energy values of feedstuffs in chicken and guinea fowl. *Indian J. Anim. Sci.,* **66**: 930-934.

Mandal, A.B. (1992). Comparative evaluation of metabolizable energy values of feedstuffs in chicken and guineafowl and energy-protein requirement of guinea keets as a meat birds. Ph. D Thesis submitted to Deemed University, IVRI, Izatnagar, India.

Mandal, A. B., Saxena, V. P. and Yadava, I. S. (1985). Utilizing rice bran and deoiled rice bran as poultry feed. (Dec. 16). *Indian Poultry Review*. 11-15.

Mandal, A. B., Tyagi, Praveen, K., Elangovan, A.V., Tyagi, Pramod, K., Kaur, S. and Johri, A. K. (2006). Comparative apparent metabolizable energy values of high, medium and low tannin varieties of sorghum in cockerel, guinea fowl and quail. *Br. Poult. Sci.*, **47**: 336-341.

Mandal, A.B., Elangovan, A.V., Tyagi, P.K., Tyagi, P.K. and Verma, S.V.S. (2002). Effect of mixed enzyme preparation on utilization of pearl millet (*Pennisetum typhoides*) in broiler chickens. *Indian J. Poult. Sci.*, **37**: 139-144.

Mandal, L., Banerjee, G. C. and Sarkar, S. K. (1974). Metabolisable energy value of deoiiled rice polish in chicks. *Indian Vet. J.*, **51(7/8)**: 527-533.

Manwar S. J., and Mandal, A B. (2009) Effect of reconstitution of sorghum with or without enzymes on production performance and immune-competence in broiler chicken. *J. Sci. Food Agric.*, **89(6)**: 998–1005.

Manwar S. J., and Mandal, A B. (2009) Effect of reconstitution of wheat with or without enzymes on growth performance and nutrient utilisation in broilers. *Br. Poult. Sci.*, **89(6)**: 521–527.

Manwar, S. J. (2007). Effect Of feed enzyme addition and reconstitution on utilization of nutritious cereals for optimum production and immunocompetence in broiler chickens. Ph. D thesis in Poultry Science, IVRI, Izatnagar, UP, India.

Manwar, S.J. and Mandal, A.B. (2008). Availability of certain nutrients in cereals reconstituted with or without enzymes for chickens. *J Sci Food Agric.*, **88**:699–706.

Mathivanan, R., Selvarja, P. and Nanjappan, K. (2006). Feeding of fermented soybean meal on broiler performance. *Int. J. Poult. Sci.*, **5(9)**: 868-872.

McCracken, J. and Quintin, G. (2000). Metabolizable energy content of diets and broiler performance as affected by wheat specific enzyme supplementation. *Br. Poult. Sci.*, **31**: 811-821.

Mercier, C. and Feillet, P. (1975). Modification of carbohydrate components by extrusion-cooking of cereal products. *Cereal Chem.*, **52**: 283-289.

Miles, R. D. and Nelson, T. S. (1974). The effect of the enzymatic hydrolysis of phytate on the available energy content of feed ingredients for chicks and rats. *Poultry Sci.,* **53(5)**: 1714-1717.

Misir, R. and Marquardt, R. R. (1978). Factors affecting rye (Secale *cereale* L.) Utilization in growing chicks. I. The influence of rye level, ergot and penicillin supplementation. *Canadian J. Anim. Sci.,* **58**: 691-701.

Mollah, Y., Bryden, W.L., Wallis, I.R., Balnare, D. and Annison, E.F. (1983). Studies on low metabolizable energy wheats for poultry using conventional and rapid assay procedures and the effect of processing. *Br. Poult. Sci.,* **24**: 81-89.

Moo-Young, M., Moreira, A. R. and Tengerdy, R. P. (1983). In The filamentous fungi, vol. 4, J. E. Smith, DE. R. Berry, B. Kristiansen, eds. Edward Arnold, London, pp. 117-144.

Moraes, A. F. (1999). Enriquecimento Protéico do Farelo de Arroz empregando Fermentação semisólida em Biorreator de Coluna com Leito Fixo. *Dissertação de Mestrado em Engenharia de Alimentos.* Fundação Universidade Federal do Rio Grande (FURG), Rio Grande.

Musharaf, N.A. and Latshaw, J.D. (1991). Effect of tannin extraction on the feeding value of grain sorghum in broiler starter diets. *Sudan J. Anim. Prod.,* **4(1)**: 53-64.

N.R.C. (1994). National Research Council. Nutrient requirements of poultry. *9^{th} Edn.,National Research Council, Nat. Acad. Press, Washington DC.*

Nagra, S.S. (1984). Effect of feeding toasted and fungal treated guar meal (*Cyamopsis tetragonaolaba*) on the utilization of nutrient in poultry. *Indian J. Poult. Sci.,* **19(3):** 201-202.

Nair, V.C., Laeamme, J. and Duvnjak, Z. (1991). Production of phytase by Aspergillus ficuum and reduction of phytic acid contentent in canola meal. *J. Sci. Food Agric.,* **54**: 355-365.

Nelson, T. S., Stephenson, E. L., Burgos, A., Floyd, J. and York, J. O. (1975). Effect of tannin content and dry matter digestion on energy utilization and amino acid digestibility of hybrid sorghum grains. *Poult. Sci.,* **54**: 1620-1623.

Nigam, P. and Singh, D. (1996). Processing of agricultural wastes in solid state fermentation for microbial protein production, *J. Sci. Ind. Res.*, **55:**373–380.

Nout, M. J. R. (1994). Fermented foods and food safety. *Food Res. Int.*, **27:** 291-301.

Nwokolo, E.N., Bragg, D.B. and Saben, H.S. (1977). A nutritive evaluation of palm kernel meal for use in poultry rations. *Trop. Sci.,* **19**: 147-154.

Odunsi, A.A.1, Akande1, T.O., Yusuph, A.S. and Salami, R.I. (2002). Comparative utilisation of high inclusion rates of four agro-industrial by-products in the diets of egg type chickens. *Arch. Zootec.*, **51**: 465-468.

Osborne, J.B. and Mendel, L.B. (1917). The use of soybeans as food. *J. Biol. Chem.,* **3**: 369-387.

Oshoma, C.E. and Ikenebomeh, M.J. (2005). Production of Aspergillus niger Biomass from Rice Bran. *Pakistan J. Nutr.,* **4 (1)**: 32-36.

Paik I.K. (2003). Application of phytase, microbial or plant origin, to reduce phosphorus excretion in poultry production. *Asian-Australas. J. Anim. Sci.,* **1**: 124-135.

Pallauf J., Rimbach G., Pippig S., Schindler B., Most E., 1994. Effect of phytase supplementation to phytate rich diet based on wheat, barley and soya on the bioavailability of dietary phosphorus, calcium, magnesium, zinc and protein in piglets. *Agribiol. Res.*, **47**: 39-48.

Pandey, A, Selvakumar, P., Soccol, C. R. and Nigam, P. (1999). Solid State Fermentation for the production of industrial enzymes. *Curr. Sci.,* **79**: 149-162.

Pandey, A. (1992). Recent process developments in solid-state fermentation. *Process Biochem.* **27**: 109-117.

Pandey, A. and Soccol, C. R. (1998), Bioconversion of biomass: a case study of ligno-cellulosics bioconversions in solid state fermentation. *Brazilian Archives Biol. Technol.,* **41 (4)**: 379-390.

Parsons, H.T. (1943). Effect of different cooking methods on soybean proteins. *J. Home. Econ.,* **35**: 211-216.

Patil, M. S. (1972). Utilization of wheat bran in the chicken starter rations. 1. Influnce of calorie/ protein ratios in the starting diets containing wheat bran. *Proceedings Australasian Poul. Sci. Convection* : 281-304.

Pileggi, V. J., Di Giorgio, J. and Wybenga, D. R. (1972). A one-tube serum uric acid method using phosphotungstic acid as protein precipitant and color reagents. *Clin. Chem. Acta.,* **37**: 141-149.

Pogaku, R., Rudravaram, R., Chandel , A. K., Rao, V. and Yim, L. Z. H. (2009). The Effect of De-Oiled Rice Bran for Single Cell Protein Production Using Fungal Cultures under Solid State Fermentation. *Int J. Food Engineering.* **5(2)**: 1.

Pointillart, A. (1991). Enhancement of phosphorus utilization in growing pigs fed phytate rich diets by using rye bran. *J. Anim. Sci.,* **69**: 1109-1115.

Prakash, O. and Singh, S. (1984). Guar meal as a poultry feed constituent. *Poult. Adviser,* **17(12):** 37-40.

Preston, C. M., McCracken, K. J. and Bedford, M. R. (2001). Effect of wheat content, fat source and enzyme supplementation on diet metabolizability and broiler performance. *Br. Poult. Sci.,* **42(5)**: 625-632.

Pym, R. A. E. and Farrell, D. J. (1977). A comparison of the energy and nitrogen metabolism of broilers selected for increased growth rate, food consumption and conversion of food to gain. *Br. Poult. Sci.,* **18**: 411-426.

Rama Rao, S. V., Shyam Sunder, G., Panda, A. K., Reddy, M. R., Raju, M. V. L. N. and Praharaj, N. K. (2002). Utilization of different millets replacing maize in coloured broiler chicken diet. *Indian J. Anim. Nutr.,* **19**: 353-358.

Ramakrishna S.V., Childyal N.P., Lonsane B.K., Ahmed S.Y. and Sreenivasa M. V. (1982). Wheat bran as substrate in solid state fermentation. *In*: Proc.Nat.Symp.Biotechnology (S.C. Jain Ed.), Chandigarh. pp. 267-283

Ravinder R., Chandel A.K., Venkateshwer L.V. and Ravindra P. (2006). Optimization of protein enrichment of de-oiled rice bran by solid state fermentation using *Aspergillus oryzae* MTCC 1846. *Int. J. Food Eng.,* **2**: 1-14.

Ravinder R., Venkateshwar Rao L. and Ravindra P. (2003) Production of SCP from de-oiled rice bran. *Food Technol. Biotechnol.,* **41**: 243–246.

Ravindran, V., Cabahug, S., Ravindran, G. and Bryden, W.L. (1999). Influence of microbial phytase on apparent ileal amino acid digestibility in feedstuffs for broilers. *Poult. Sci.,* **78**: 699-706.

Reddy, C.V., Jensen, L.S., Merrill, L.H. and McGinnis, J. (1961). Influence of pelleting on metabolizable and productive energy content of a complete diet for chicks. *Poult. Sci.,* **40**: 1446.

Reddy, N. R. and Pierson, M. D. (1994). Reduction in antinutritional and toxic components in plant foods by fermentation. *Food Res. Int.,* **27**: 281-289.

Reddy, N. R., Sathe, S. K. and Salunkhe, D. K. (1982). Phytases in legumes and cereals. *Adv. Food Res.*, **28**: 1–92.

Reddy, N.R. and Pierson, M.D. (1994). Reduction in anti-nutritional and toxic components in plant foods by fermentation. *Food Res. Int.,* **27:** 281-289.

Reddy, N.V., Rao, D.R. and Sunki, G.R. (1979). Comparison of maize, wheat and triticale in broiler diets. *Br. Poult. Sci.,* **20**: 357-62.

Reddy, V. R. and Vaidya, S. V. (1973). Feed composition tables for poultry feeds. *Poult. Adviser.* **VI (2)**: 29-38.

Reece, F.N., Lott, B.D. and Deaton, J.W. (1985). The effect of feeding form, grinding method, energy level and gender on broiler performance in a moderate (21^{o}C) environment. *Pout. Sci.,* **64 (10)**: 1834-1839.

Reisen, W.H., Clandinin, D.R., Elvehjem, C.A. and Cravens, W.W. (1997). Liberation of essential amino acids from raw properly heated and over heated soybean oil meal. *J. Biol. Chem.*, **167**: 143-150**.**

Rotter, B.A., Marquardt, R.R., Guenter, W., Biliaderis, C. and Newman, C.W. (1989). *In vitro* viscosity measurements of barley extracts as an indicator of growth responses in chicks fed barley-based diets supplemented with a fungal enzyme preparation. *Canadian J. Anim. Sci.*, **69**: 431–439.

Rudravaram, R., C. Anuj Kumar, V. R. Linga, and Pogaku, R. (2006). Optimization of protein enrichment of deoiled rice bran by solid state fermentation using *Aspergillus oryzae* MTCC 1846. *Int. J. Food Engineering*, **2 (4)**: Article 1.

Saima, M. A. and Hasmi, A. S. (1999). Bioconversion of wheat bran to biomass protein through metabiosis and its biological evaluation in broiler chicks. *Int. J. Agric. Biol.*, **1(1/2)**:39-41.

Saima, M. A. Jabbar, T. N. Pasha and Khan, M. Z. U. (2007). Production and biological evaluation of microbial biomass by fermentation of melon-peels with *Aspergillus niger*. International Tropical Animal Nutrition Conference (TROPNUTRICON-2007)Volume II.

Saki, A.A. (2005). Effect of Wheat and Barley Viscosity on Broiler Performance in Hamadan Province. *Inernational. J. Poult. Sci.*, **4(1)**: 7-10.

Sannamani, P. G. (2002). Feeding value of sorghum grains vis-a-vis yellow maize for broiler chicks. *M.V.Sc. Thesis, IVRI, Izatnagar.*

Sannamani, P., Tyagi, Pramod Kr., Tyagi, P. K. Shastry, V. R. B., Elangovan, A. V. and Mandal, A. B. (2010). Effect of feeding of low and high tannin sorghum on carcass quality, skeletal status and histopathology of broiler chicken. *Indian J. Poult. Sci.*, **45 (2)** : 133-140.

Sastry, V.R.B., Kamra, D. N. and Pathak, N. N. (1999). Laboratory Manual of Animal Nutrition. *CAS in Anim. Nutr., IVRI, Izatnagar.*

Sayre, R.N., Earl, L., Kratzer, F.H. and Saunders, R.M.C. (1988). Effect of diets containing raw and extrusion-cooked rice bran on growth and efficiency of food utilization of broilers. *Br. Poult. Sci.*, **29 (4)**: 815-823.

Scheele, C.W. and Kwakernaak, C.K. (1996). Additives improving the utilization of proteins, fats and carbohydrates in poultry diets, *In Proc. XX World's. Poult. Cong., New Delhi, India,* pp. 113-117.

Schutte, J.B., de Jong, J. and Langhout, D.J. (1995). Effect of xylanase supplimentation to wheat-based diet in broiler chicks in relation to dietary factors. ln: Proc.2nd European Symposium on Feed Enzymes. 25-27 Oct. Noordwijkerhout, Netherlands, TNO Nutr. Food Res. Inst., Zeist, Netherlands, pp. 95-101.

Scott, M. L., Nesheim, M.C. and Young, R.J. (1982). Nutrition of the Chicken. 3rd ed. M.L. Scott and Associates, Ithaca, New York .

Segueilha, L., Moulin, G. and Galzy, P. (1993). Reduction of phytate content in wheat bran and landless cotton flour by Schwanniomyces castellii. *J. Agric. Food Chem.*, **41**: 2451-2454.

Sen, K. C., Ray, S. N. and Ranjhan, S. K. (1978). Nutritive value of Indian cattle feeds and feeding of animals. ICAR, New Delhi.

Shah P., Bhavsar, K., Soni, S. K. and Khire J. M. (2009). Strain improvement and up scaling of phytase production by *Aspergillus niger* NCIM 563 under submerged fermentation conditions. *J Ind Microbiol Biotechnol.,* **36**:373–380

Sharda, D. P. and Thakur, R S. (1977). Effect of feeding sorghum as a replacer of maize on carcass yield and internal body organs of broilers. *Indian J. Poult. Sci.,* **12**: 25-28.

Shojaosadati, S. A., Faraidouni, R., Madadi-Nouei, A. and Mohamadpour, I. (1999). Protein enrichment of lignocellulosic substrates by solid state fermentation using Neurospora sitophila. *Resources, Conservation and Recycling,* **27:** 1-2, 73-87.

Sibbald, I.R. and Slinger, S.J. (1963). A biological assay for metabolizable energy in poultry feed ingredients together with findings which demonstrate some of the problems associated with the evaluation of fats. *Br. Poult. Sci.,* **42**: 313-324.

Siegel, P. B. and Gross, W. B. (1980). Production and persistence of antibodies in chickens to sheep erythrocytes. I. Directional selection. *Poult. Sci.,* **59**: 1-5.

Sikka, S. S. and Chawla, K. S. (2002). Influence of ezyme supplementation to wheat and rice kani based diets on the performance of broilers. *Anim. Nutri. Feed Tecnol.* **2:** 11-18.

Silva, M. A.; Sanches, C.; Amante, E. R. (2001), Farelo de arroz – composição e propriedades. Revista Óleos and Grãos, jul-ago, 34-42.

Silveira, C. M. and Badiale-Furlong, E. (2009). Sperathe Effects of Solid-state Fermentation in the Functional Properties of Defatted Rice Bran and Wheat Bran. *Braz. Arch. Biol. Technol.,* **52(6)**: 1555-1562.

Simons, P.C.M., Versteegh, H.A.J., Jongbloed, A. W., Kemme, P. A., Slump, P., Bos, K. D., Wolters, M.G.E., Beudeker, R. F. and Verschoor, G. J. (1990). Improvement of phosphorus availability by microbial phytase in broilers and pigs. *Br. J. Nutr.*, **64**: 525–540.

Singh, A. S. (1997) Studies on effect of replacement of wheat bran with deoiled rice bran from the maintenance diet of adult cross-breed cows. M. V. Sc Thesis submitted to Deemed University, IVRI, Izatnagar, UP. India.

Snedecor, G. W. and Cochran, W. G. (1989). Statistical Methods. 8th Edition. Iowa State University Press, Ames Iowa, USA.

Snedecor, G. W. and Cochran, W. G. (1989). Statistical methods. The lowa State University Press, Ames, Iowa, USA.

Soaad, H., Bayati, A. L. and Al-Rayess, H. (1981). The chemical composition of Iraqui rice and rice by-products. *J. Food Sci. Technol.*, **18**: 40-44.

Solomon, B.O., Amigun, B., Betikue, T.V., Ojumu, T., Layokun, S.K. (1999). Optimization of cellulase production by *Aspergillus* flavus *Linn*. Isolates NSPR 101 grown on bagasse. *JNSCHE*., **18:** 61-68.

Steenfeldt, S., Mullertz, A. and Jensen, J.F. (1998). Enzyme supplementation of wheat-based diets for broilers. 1. Effect on growth performance and intestinal viscosity. *Anim. Feed Sci. and Technol.,* **75:** 27-43.

Steiner T., Mosenthin R., Zimmermannb B., Greiner R. and Roth S. (2007). Distribution of phytase activity, total phosphorus and phytate phosphorus in legume seeds, cereals and cereal by-products as influenced by harvest year and cultivar. *Anim. Feed Sci. Technol.*, **133**: 320-334.

Steinkraus, K. H. (1994). Nutritional significance of fermented foods, *Food Res. Int.,* **27**: 259-268.

Svihus, B. and Heltland, H. (2001). Ileal digestibility in growing broiler chickens fed on a wheat based diet is improved by mash feeding, dilution with cellulose or whole wheat inclusion. *Br. Poult. Sci.*, **42**: 633-637.

Swaminathan, M. and Bhagawan, R. K. (1969). Our Food, 6th Ed., pp. 49-50. Ganesh & Co., Madras.

Swe, K.H. (2004). Evaluation of solid state fermentation by Aspergillus niger to improve the nutritive value of palm kernel cake for broilers. Doctor of Philosophy Thesis, University Putra Malaysia.

Sze-Tao, K.W.C. and Sathe, S.K. (2000), Functional properties and in vitro digestibility of almond *(Prumus dulcis L.)* protein isolate. *Food Chem.*, **69**(2), 153-160.

Takemasa, M. and Murakmi, H. (1995). Reduction of phosphorus excretion of chicks by usage of barley or wheat. *Jap. Poult. Sci.*, **32**: 342-349.

Talpatra, S. K., Roy, S. C. and Sen, K. C. (1940). Estimation of phosphorus, choline, calcium, magnesium, sodium, potassium in feeding stuffs. *Indian J. Vet. Sci.*, **10**:243-247.

Tyagi, P. K., Elangovan, A. V., Mandal, A. B., Tyagi, P. K., Kaur, S. and Johri, A. (2003). Effects of feeding low tannin sorghum grain to broiler chickens. *Indian J. Anim. Nutr.*, **20**: 322-326.

Tyagi, P.K., Tyagi, P.K. and Verma, S.V.S. (1998). Phytate phosphorus content of some common poultry feed stuffs. *Indian J. Poult. Sci.*, **33(1)**: 86–88.

Tyagi, Pramod K., Tyagi, Praveen K. and Verma, S.V.S. (1997). Effect of water soaking of wheat on phytate content and utilization by chicks. *Indian J. Poult. Sci.*, **32 (3)**: 216-219.

Tyagi, Praveen K., Tyagi, Pramod K. and Verma, S.V.S. (1998). Effect of water soaking of wheat-bran on phytate phosphorus autolysis and its feeding value to chicks. *Indian J. Anim. Sci.*, **68 (7)**: 669-671.

Union budget and Economic Survey, GOI 2010-11 Economic Survey, Union Budget of India GOI (2010-11). Agriculture and Food Management. Pp 187-216. *Website: http://indiabudget.nic.in*

Usha, A., Sripriya, G. and Chandra, T.S. (1996). Effect of fermentation on primary nutrients in finger millet (*Eleusine coracane*). *J. Agric. Food Chem.*, **44**: 2616-2619.

Vahjen, W., Gläser, K. , Schäfer, K. and Simon, O. (1998). Influence of xylanase-supplemented feed on the development of selected bacterial groups in the intestinal tract of broiler chicks. *J. Agric. Sci.*, **130**: 489-500

Van der Klis, J.D., Kwakernaak, C. and De Wit, W. (1995a). Effects of endoxylanase additions to wheatbased broiler diets on physico-chemical chyme conditions and mineral absorption in broilers. *Anim. Feed Sci. Tech.,* **51**: 15-27.

Van der Klis, J.D., Scheele, C.W. and Kwakernaak, C. (1995b). Wheat characteristics related to its feeding value and to the response of enzymes. In: Proc. 10th European Symposium on Poultry Nutrition, Antalya (Turkey).

Van der Zijpp, A. J., Frankena, K., Boneschanscher, J. and Nieuwland, M. G. (1983). Genetic analysis of primary and secondary immune responses in the chicken. *Poult. Sci.,* **62**: 565-572.

Van Soest, P.J. (1963). Use of detergents in the analysis of fibrous feeds. II. A rapid method for the determination of fibre lignin. *J. Asso. Agri. Chem.,* **46**: 829-835.

Veldman, A. and Vahl, H.A. (1994). Xylanase in broiler diets with differences in characteristics and content of wheat. *Br Poultry. Sci.,* **35**: 537–550.

Verma, D. N. (1995). A text book of Animal Nutrition. First edn., Kalyani publisher, Rajendra nagar, Ludhiana – 141008, India.

Verma, S. V. S. and McNab, J. M. (1985). Effect of feeding guar gum to chicks on utilization of nutrients. *Ind. J. Anim. Nutr.,* **2(2)**: 69-74.

Verma, S. V. S. and Shrivastava, H. P. (1989-90). Nutritional evaluation and upgrading the feeding value of agro-industrial residues for poultry. Apple pomace and deoiled rice bran. Annual Sci. Rep. AN & FT, CARI, Izatnagar. pp: 11-29.

Verma, S. V. S., Shrivastava, H. P. and Tyagi, P. K. (1990-91). Nutritional evaluation and upgrading the feeding value of deoiled rice bran for poultry. *Annual Sci. Rep.* AN& FT, CARI, Izatnagar. pp: 9-15.

Verma, S.V.S. and McNab, J.M. (1982). Guar meal in diets for broiler chickens. *Br. Poult. Sci.,* **23:** 95-105.

Verma, S.V.S. and Singh, R. (1985). Nutritional value of fermented guar meal for broilers. *World Rev. Anim. Prod.,* **21(2):** 47-50.

Viveros, A., Brenes, A., Pizarro, M. and Castano, M. (1994). Effect of enzyme supplementation of a diet based on barley, and autoclave treatment, on apparent digestibility, growth performance and gut morphology of broilers. *Anim. Feed Sci. Technol.,* **48**: 237–251.

Viveros, A., Brenes, C. A., Canales, R. and Lozano, A. (2000). Phytase and acid phosphatase activities in plant feedstuff. *J. Agri. Food Chem.,* **48**: 4009-4013.

Vohra, P. and Kratzer, F. H. (1964a). The use of guar meal in chicken rations. *Poult. Sci.,* **43:** 502-03.

Vohra, P. and Kratzer, F. H. (1964b). Growth inhibiting effect of certain polysaccharides for chickens. *Poult. Sci.,* **43:** 1164-70.

Wahal, C. K. and Saxena, K. K. (1979). Qualitative and quantitative estimation of amino acids of different feeds and fodder of Indian origin for the evaluation of protein quality. *Annual Scientific Report of the Division of Animal Nutrition (Indian Veterinary Research Institute),* Project No. 2. 18, p. 36.

Wan, H. F., Chen, W., Qi, Z. L., Peng, P. and Peng, J. (2009) .Prediction of true metabolizable energy from chemical composition of wheat milling by-products for ducks, *Poult. Sci.,* **88**:92–97.

Wang, H. L., Vespa, J. B. and Hesseltine, C. W. (1972). Release of bound trypsin inhibitors in soybeans by *Rhizopus oigosporus*. *J. Nutr.,* **102**: 1495-1499.

Warren, B.E. and Farrell, D.J. (1990a). The nutritive value of full-fat and defatted Australian rice bran. I. Chemical composition. *Anim. Feed Sci. Technol.,* **27:** 219–228.

Warren, B.E. and Farrell, D.J. (1990b). The nutritive value of full-fat and defatted rice bran. II. Growth studies with chickens, rats and pigs. *Anim. Feed Sci. Technol.,* **27:** 229–246.

Warren, B.E. and Farrell, D.J. (1990c). The nutritive value of full-fat and defatted rice bran III. The apparent digestible energy content of defatted rice bran in rats and pigs and the metabolizability of energy and nutrients in defatted and full-fat bran in chickens and adult cockerels. *Anim. Feed Sci. Technol.,* **27:** 247–257.

Warren, B.E. and Farrell, D.J. (1990d). The nutrient value of full-fat and defatted rice bran. IV. Egg production of hens on diets with defatted rice bran. *Anim. Feed Sci. Technol.,* **27:** 259–268.

Warren, B.E. and Farrell, D.J. (1991). The nutritive value of full-fat and defatted Australian rice bran. V. The apparent retention of minerals and apparent digestibility of amino acids in chickens and adult cockerels with ileal cannulae. *Anim. Feed Sci. Technol.,* **34:** 323–347.

White, T. W. (1965). Rice bran in beef cattle fattening rations. *Louisiana Agricultural Experimental Station, Bulletin* 600.

Wiseman, J. (2000).Correlation between physical measurements and dietary energy values of wheat for poultry and pigs. *Anim. Feed Sci. Tec.,* **84**: 1-11.

Wyatt, C.L., Bedford, M.R. and Waldron, L.A. (1999). Role of enzymes in reducing the variability in nutritive value of maize using the ileal digestibility method. *In Proc. Aust. Poult. Sci. Symp.,* **11**: 108-111.

Wybenga, D. R., Pileggi, V. J., Dirstine, P. H.and Di Giorgio, J. (1970). Direct manual determination of serum total cholesterol with a single stable reagent. *Clin. Chem.,* **16**: 980-1004.

Yao, J.H., Han, J.C., Wu, S.Y., Xu, M., Zhong, L.L., Liu, Y.R and Wang, Y.J. (2007). Supplemental wheat bran and microbial Phytase couldreplace inorganic phosphorus in laying hen diets. *Czech J. Anim. Sci.,* **52(11)**: 407-413.

Yasar, S. and Forbes, J. M.(1999). Performance and gastro-intestinal response of broiler chickens fed on cereal grain-based feeds soaked in water. *Br. Poult. Sci.,* **40**: 65-76.

Zadrazil, F. and Puniya, A. K. (1995). Studies on the effect of particle size on solid-state fermentation of sugarcane bagasse into animal feed using white-rot fungi. *Bioresour. Technol.,* **54**:85–87.

Zamora, R.G. and Veum, T.L. (1979). Whole soybeans fermented with *Aspergillus oryzae* and *Rhizopus oligosporus* for growing pig. *J. Anim. Sci.,* **48(1):** 63-68.

Zanini, S. F. and. Sazzad M.H. (1999). Effects of microbial phytase on growth and mineral utilization in broiler fed on maize soybeanbased diets. *Br. Poult. Sci.,* **40:**348-352.

Zeisel, S.H., Mar, M.H., Howe, J.C and Olden, J.M. (2003). Concentration of choline-containing compounds and betaine in common foods. *J.Nutr.,* **133**: 2918-2919.

Zhou S., Yomano, L.P., Shanmugam, K.T. and Ingram, L.O. (2005). Fermentation of 10% (w/v) Sugar to D-Lactate by Engineered Escherichia coli-B. *Biotechnol. Letters,* **27**: 23-24.

Zhuge, Q., Yan, Z., Klopfenstein, C.F. and Behnke, K.C. (1990). Nutritional value of sorghum grain depends on processing. *Feedstuffs,* **42**: 18-20.

Zombade, S.S. and Ichhponani, J.S. (1983). Nutritive value of raw, parboiled, stabilized and deoiled rice bran for growing chicks. *J. Sci. Food Agric.*, **34**: 783-786.

Zombade, S.S., Lodhi, G.N. and Ichhponani, J.S. (1977). A note on reassessment of metabolizable energy content of rice bran for poultry. *Indian J. Anim. Sci.,* **47(1)**: 51–52.

❖❖❖

Appendix...

Appendix

ANALYSIS OF VARIANCE

Table no.	d.f.		Mean sum of squares (MSS)	
	Between treatment	Error	Between treatment	Error
Table 4.1.1a: Chemical composition (%DM) of WB				
DM loss	2	12	15.313	1.032**
CP	3	8	15.503	0.741**
CF	3	8	9.489	0.568**
EE	3	8	1.631	0.045**
NFE	3	8	1.184	2.419^{NS}
GE	3	8	14380.750	1158.583**
Table 4.1.2a: Mineral composition (%DM) of WB				
TA	3	8	2.021	1.532 ^{NS}
AIA	3	8	0.353	0.011**
Ca	3	8	0.006	0.002 ^{NS}
P	3	8	0.048	0.003**
Table 4.1.3a: Aflatoxin and urease activity of WB				
Aflatoxin	3	8	0.0000	0.0000 ^{NS}
Urease	2	6	0.0000	0.0000 ^{NS}
Table 4.1.4a: Chemical composition (%DM) of DORB				
DM loss	2	12	24.975	13.553 ^{NS}
CP	3	8	10.347	1.117**
CF	3	8	21.593	0.440**
EE	3	8	0.235	0.014**
NFE	3	8	18.700	1.478**
GE	3	8	5668.972	752.917**
Table 4.1.5a: Mineral composition (%DM) of DORB				
TA	3	8	18.575	1.232**
AIA	3	8	0.667	0.044**
Ca	3	8	0.017	0.002**
P	3	8	0.058	0.003**
4.1.6a: Aflatoxin and urease activity of DORB				

Aflatoxin	3	8	0.0000	0.0000 NS
Urease	2	6	0.0000	0.0000 NS

Table 4.2.2a: Intake of feed, CP and AMEn and % excreta DM of WB

Source of variance	d.f.	Mean sum of squares (MSS)			
		Feed intake	CP intake intake	AME_n intake	DM excreta
Interaction	3	0.011 NS	0.485 NS	21.640 NS	30.148 NS
WB types	3	0.098**	2.873**	88.011**	47.961**
Inclusion levels	1	0.011**	194.891**	13558.610**	27.407**
Error	40	0.000	0.000	9.801	0.268

Table 4.2.3a: $AMEn^{mc}$, GEM and DMM of test diets and WB

Source of variance	d.f.	Diet			WB	
		$AMEn^{mc}$	GEM	DMM	AMEn	DMM
Interaction	3	2499.376 NS	19.911**	30.340**	2481.583 NS	35.771**
WB types	3	12458.946**	82.919**	43.299**	133203.472**	55.360**
Inclusion levels	1	1654230.615**	134.804**	2.770**	10860.083 NS	32.571**
Error	40	1137.982	0.536	0.334	17333.717	0.317

Table 4.2.4a: Intake of feed, CP and AMEn and % excreta DM of DORB

Source of variance	d.f.	Mean sum of squares (MSS)			
		Feed intake	CP intake intake	AME_n intake	DM excreta
Interaction	3	2.245 NS	0.454 NS	35.959 NS	96.344 NS
DORB types	3	0.300 NS	0.977**	34.934 NS	25.434*
Inclusion levels	1	2.245**	193.925**	3836.333**	187.586**
Error	40	0.000	0.000	22.616	0.366

Table 4.2.5a: $AMEn^{mc}$, GEM and DMM of test diets and DORB

Source of variance	d.f.	Diet			DORB	
		$AMEn^{mc}$	GEM	DMM	AMEn	DMM
Interaction	3	1465.910 NS	61.181**	108.734**	36740.139 NS	114.241**
DORB types	3	6296.743 NS	6.498*	30.186**	97029.500 NS	29.804**
Inclusion levels	1	538692.188**	0.667NS	244.307**	40716.750 NS	222.310**
Error	40	2795.246	1.616	0.442	46210.933	0.435

Table no.	d.f.		Mean sum of squares (MSS)	
	Between treatment	Error	Between treatment	Error

Table 4.2.6a: Effects of fermentation of WB and DORB on availability of certain nutrients

Wheat bran (WB) types

IVPPD	3	8	20.154	2.795*
ACHOS	3	8	84.405	3.288**

Phytate P (%)	3	8	0.020	0.000**
Phytate P (%, TP)	3	8	455.657	
NPP (%)	3	8	0.124	0.000**
NPP (%, TP)	3	8	1348.828	0.420**
ADF (%, DM)	3	8	2.183	0.487*
De-oiled rice bran (DORB) types				
IVPPD	3	8	61.079	3.028**
ACHOS	3	8	60.002	3.615**
Phytate P (%)	3	8	0.182	0.000**
Phytate P (%, TP)	3	8	550.993	0.170**
NPP (%)	3	8	0.112	0.000**
NPP (%, TP)	3	8	550.993	0.170**
ADF (%, DM)	3	8	2.567	0.415*
Table 4.3.1a: Effect of feeding FWB on body weight gain (g/b) of broilers				
1 wk	7	248	1658.049	642.372*
2 wk	7	248	2777.080	543.973**
3 wk	7	248	9441.938	1540.998**
4 wk	7	248	4962.917	1723.865**
5 wk	7	248	9327.676	2975.194**
6 wk	7	248	10939.087	5534.204 NS
0 – 3 wk	7	248	16192.482	4356.071**
3 – 6 wk	7	248	9167.040	12364.887 NS
0 – 6 wk	7	248	6969.473	16989.685 NS
Table 4.3.2a: Effect of feeding FWB on feed intake (g/b) of broilers				
1 wk	7	24	129.911	296.396 NS
2 wk	7	24	271.696	150.917 NS
3 wk	7	24	2555.853	822.26*
4 wk	7	24	1897.268	857.042 NS
5 wk	7	24	3273.888	1583.219 NS
6 wk	7	24	6422.746	1942.885*
0 – 3 wk	7	24	2588.674	2302.823 NS
3 – 6 wk	7	24	9077.853	5636.531 NS
0 – 6 wk	7	24	5397.424	6622.948 NS
Table 4.3.3a: Effect of feeding FWB on FCR of broilers				
1 wk	7	24	0.019	0.014 NS
2 wk	7	24	0.010	0.006 NS
3 wk	7	24	0.009	0.011 NS
4 wk	7	24	0.004	0.007 NS
5 wk	7	24	0.015	0.022 NS
6 wk	7	24	0.007	0.007 NS
0 – 3 wk	7	24	0.005	0.004 NS
3 – 6 wk	7	24	0.006	0.004 NS
0 – 6 wk	7	24	0.004	0.003 NS

Table 4.3.5a. Effect of feeding FWB on protein efficiency (PE) and energy efficiency (EE) of broilers

PE 0 – 3 wk	7	24	0.000	0.000 NS
PE 3 – 6 wk	7	24	0.000	0.000 NS
PE 0 – 6 wk	7	24	0.000	0.000 NS
EE 0 – 3 wk	7	24	0.042	0.030 NS
EE 3 – 6 wk	7	24	0.041	0.040 NS
EE 0 – 6 wk	7	24	0.034	0.023 NS

Table 4.3.6a. Effect of feeding FWB on feed cost of broiler production (Rs.)

Cost/kg gain				
0-3wk	7	24	3.239	1.044*
Cost/kg gain				
3-6 wk	7	24	2.654	1.396 NS
Cost/kg gain				
0-6wk	7	24	2.731	0.793*
Feed cost/kg BWT	7	24	2.878	0.843*
Cost/kg meat	7	24	3.572	1.347*

Table 4.3.7a. Effect of feeding FWB on nutrient utilization by broilers

N-retained	7	24	0.127	0.02**
N-retention	7	24	57.228	11.506**
Excreta DM	7	24	21.379	19.159 NS
DMM	7	24	3.189	0.524**
GEM	7	24	1.339	0.489*
AMEn	7	24	5998.429	944.125**
Difference	7	24	6.302	1.052**
Ca retention	7	24	7.661	0.81**
P retention	7	24	12.466	0.753**

Table 4.3.8a: Effect of feeding FWB on carcass traits (% live weight) of broilers

Blood loss	7	56	0.965	0.459 NS
Feather loss	7	56	4.309	0.608**
Eviscerated yield	7	56	8.273	2.471**
Edible yield	7	56	7.002	1.603**
Giblet yield	7	56	0.318	0.283 NS
Ready-to-cook	7	56	6.809	1.770**
Abd.fat pad	7	56	0.125	0.065 NS

Table 4.3.9a: Effect of feeding FWB on cut-up parts (% eviscerated weight) of broilers

Breast	7	56	3.771	2.563NS
Drumstick	7	56	1.818	0.406**
Thigh	7	56	2.682	0.657**
Back	7	56	3.005	1.687 NS
Neck	7	56	0.477	0.446 NS
Wings	7	56	1.172	0.363**

Table 4.3.10a: Effect of feeding FWB on vital organs and immune organs (% live weight)

of broilers

Gizzard	7	56	0.071	0.120 NS
Heart	7	56	0.006	0.004 NS
Liver	7	56	0.166	0.096 NS
Spleen	7	56	0.006	0.002*
Bursa	7	56	0.013	0.014 NS

Table 4.3.11a: Effect of FWB on certain serum (mg/100ml), immune parameters and caeca microbial count (cfu/g) of broilers

Cholesterol	7	56	527.850	468.363 NS
Triglycerides	7	56	77.507	76.592 NS
Uric acid	7	56	3.150	2.952 NS
CMI response	7	56	0.004	0.001**
HA titer	7	56	3.891	2.033 NS
Caeca microbes TPC	7	16	517.042	110.167**

Table 4.3.12a: Effect of feeding fermented wheat bran on sensory attributes of meat

Appearance	7	16	0.756	1.917 NS
Texture	7	16	0.308	0.990 NS
Juiciness	7	16	1.641	0.969 NS
Flavour	7	16	0.357	0.583 NS
Tenderness	7	16	1.399	0.646 NS
Acceptability	7	16	0.213	0.948 NS

Table 4.4.1a : Effect of feeding FDORB on body weight gain (g/b) of broilers

1 wk	7	248	580.522	294.796 NS
2 wk	7	248	3449.266	1008.017**
3 wk	7	248	3930.026	2588.875 NS
4 wk	7	248	6961.647	3859.283 NS
5 wk	7	248	6165.691	3940.449 NS
6 wk	7	248	14718.587	6371.560*
0 – 3 wk	7	248	12243.982	7115.212 NS
3 – 6 wk	7	248	14168.313	18923.272 NS
0 – 6 wk	7	248	31348.518	32622.292 NS

Table 4.4.2a. Effect of feeding FDORB on feed intake (g/b) of broilers

1 wk	7	24	195.746	226.323 NS
2 wk	7	24	522.982	140.500**
3 wk	7	24	2281.996	920.219*
4 wk	7	24	2376.210	1763.010NS
5 wk	7	24	8105.339	1654.813**
6 wk	7	24	8621.786	1652.063**
0 – 3 wk	7	24	4669.138	2359.010 NS
3 – 6 wk	7	24	45105.643	9042.604**
0 – 6 wk	7	24	68989.357	16303.771**

Table 4.4.3a. Effect of feeding FDORB on FCR (g/b) of broilers

1 wk	7	24	0.030	0.031 NS
2 wk	7	24	0.069	0.014**
3 wk	7	24	0.032	0.011*
4 wk	7	24	0.084	0.022**
5 wk	7	24	0.161	0.044**
6 wk	7	24	0.576	0.146**
0 – 3 wk	7	24	0.029	0.010*
3 – 6 wk	7	24	0.112	0.018**
0 – 6 wk	7	24	0.069	0.008**

Table 4.4.5a. Effect of feeding FDORB on protein efficiency (PE) and energy efficiency (EE) of broilers

PE 0 – 3 wk	7	24	0.001	0.000*
PE 4 – 6 wk	7	24	0.004	0.001**
PE 0 – 6 wk	7	24	0.003	0.000**
EE 0 – 3 wk	7	24	0.233	0.079*
EE 3 – 6 wk	7	24	0.951	0.151**
EE 0 – 6 wk	7	24	0.581	0.068**

Table 4.4.6a. Effect of feeding FDORB on feed cost of broiler production (Rs.)

Cost/kg gain				
0-3wk	7	24	10.705	2.762**
Cost/kg gain				
3-6 wk	7	24	39.029	4.707**
Cost/kg gain				
0-6wk	7	24	24.434	2.204**
Feed cost/kg BWT	7	24	22.704	2.034**
Cost/kg meat	7	24	39.185	3.757**

Table 4.4.7a. Effect of feeding FDORB on nutrient utilization by broilers

N-retained	7	24	0.081	0.023*
N-retention	7	24	34.393	24.003 NS
Excreta DM	7	24	114.232	28.568**
DMM	7	24	16.907	0.552**
GEM	7	24	9.117	0.365**
AMEn	7	24	599.196	649.063 NS
Difference	7	24	0.880	0.664 NS
Ca retention	7	24	21.910	1.011**
P retention	7	24	56.726	1.149**

Table 4.4.8a: Effect of feeding FDORB on carcass traits (% live weight) of broilers

Blood loss	7	56	0.882	0.952 NS
Feather loss	7	56	5.741	1.457**
Eviscerated yield	7	56	2.077	4.774 NS
Edible yield	7	56	2.238	4.436 NS
Giblet yield	7	56	0.242	0.198 NS

Ready-to-cook	7	56	1.829	4.641 NS
Abd.fat pad	7	56	0.167	0.104 NS

Table 4.4.9a: Effect of feeding FDORB on cut-up parts (% eviscerated weight) of broilers

Breast	7	56	2.629	2.474 NS
Drumstick	7	56	1.871	1.088 NS
Thigh	7	56	0.862	1.231 NS
Back	7	56	0.851	1.851 NS
Neck	7	56	0.413	0.438 NS
Wings	7	56	0.707	0.433 NS

Table 4.4.10a: Effect of feeding FDORB on vital organs and immune organs (% live weight) of broilers

Gizzard	7	56	0.093	0.090 NS
Heart	7	56	0.001	0.004 NS
Liver	7	56	0.098	0.066 NS
Spleen	7	56	0.002	0.003 NS
Bursa	7	56	0.008	0.007 NS

Table 4.4.11a: Effect of FDORB on certain serum (mg/100ml), immune parameters and caeca microbial count (cfu/g) of broilers

Cholesterol	7	56	217.604	210.050 NS
Triglycerides	7	56	1.078	0.389*
Uric acid	7	56	221.243	79.826*
CMI response	7	56	0.007	0.002**
HA titer	7	56	2.837	2.132 NS
Caeca microbes TPC	7	16	209.641	70.500*

Table 4.4.12a: Effect of feeding FDORB on sensory attributes of meat

Appearance	7	16	1.113	1.229 NS
Texture	7	16	0.713	1.719 NS
Juiciness	7	16	2.327	1.417 NS
Flavour	7	16	1.089	0.583 NS
Tenderness	7	16	0.286	0.750 NS
Acceptability	7	16	0.284	0.781 NS

** P<0.01; * P<0.05; NS- Non-significant

Vitae

Name of the author	:	**Dr. Chintamani Pandurang Munj**
Parent's name	:	Mr. Pandurang Vasudev Munj Mrs. Rukhmini Pandurang Munj
Date of birth	:	31st August, 1982
Permanent address	:	S/o Mr. P. V. Munj
	:	Mangaon, Kudal,
		Distt. Sindhudurg, Maharashtra - 416 519.
		Ph. No. 02362 236549 (Resi.).
E-mail	:	munjchintamani@gmail.com
	:	chintamanimunj@yahoo.co.in

Educational qualifications :

1. B.V.Sc. & A.H. (2005), Bombay Veterinary College, Maharashtra Animal & Fishery Sciences University, Nagpur, Maharashtra.
2. M.V.Sc. (2008), Bombay Veterinary College, Maharashtra Animal & Fishery Sciences University, Nagpur, Maharashtra.
3. Ph.D. (2011), Indian Veterinary Research Institute, Izatnagar, India.

Fellowship awarded :

1. SRF or IVRI Institute Fellowship- 2008 for PhD degree in Poultry Science.
2. Awarded ICAR NET 2009.

Professional membership :

1. Life member, Indian Journal Poultry Science Journal, Indian Poultry Science Association, CARI, Izatnagar.
2. Life member Bombay Veterinary College Alumni Association Journal.
3. Life member, Maharashtra State Veterinary Council, Maharashtra.
4. Life member, Veterinary Council of India.

Publications :

National	:	03
Research abstract	:	07
Technical articles	:	10
Magazine articles	:	02

(Chintamani Pandurang Munj)