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Review article

Injectable anesthesia in farm animals

Dr. Lionel Dawson
Oklahoma State University

Introduction

Injectable anesthesia in farm animals been used on a routine basis in academia and in clinical practice in the United States of America. As an ambulatory clinician, the author has used various pharmaceuticals and combinations for sedation and short term generalized anesthesia, on farm animals in performing various techniques, clinical procedures,major and minor surgeries in the clinic and on the farm. There are many physical and mental challenges in dealing with farm animals when safely restraining or immobilizing them for any surgical or non-surgical procedures performed. The main goal is to effectively control the animal without injuring the /to subject/animal or the personnel involved. In most cases, for performing physical examination or minor surgery a chute, crush, ropes, or local anesthesia is sufficient to accomplish the task. However, on certain situations, sedatives, dissociative, and systemic analgesics can provide the mental distraction needed to reduce reflex reactions and override learned behaviors. This article will review some of the common uses of sedation and anesthesia in performing both minor and major surgeries using injectable anesthetic drugs in farm animals.

Pre-anesthetic Considerations

Important pre-anesthetic considerations involve thorough evaluation of patient health status and demeanor. This examination helps determine if the patient is a suitable candidate for field anesthesia and may reduce liability should anesthetic complications occur. Major anesthetic challenges peculiar to ruminants include restriction of ventilation due to rumen size, continuous flow of saliva (volumes up to 16 and 200 litres/day in small ruminants and adult cattle respectively), regurgitation and aspiration of rumen content, if the animal is not properly fasted, and patient size. Functioning ruminants should be ideally fasted prior to general anesthesia according to the following guidelines: adult cattle (48-hrs. for hay, 36 hrs. for grain or concentrates and 12 hrs. for water), and small ruminants and camelids (24 to 48 hrs. for hay, 12 hrs. for water). Young livestock on a milk diet (<1.5 - 2 months of age) and swine should be fasted for ~ 12 hrs. (feed & water).

If a surgery or procedure necessitating a recumbent position performed out in the field, the ground surface should ideally be soft to help protect against injury during induction and recovery. Large ruminants are more difficult to physically control and require a somewhat larger “safety zone”. Except for a handful of cases, they typically do not attempt to stand up until they are well awake and functional. Good footing is the primary requirement for achieving a good recovery: an open grassy area is ideal. A stall or pen deeply bedded with shavings,

straw or sand provides a good surface, but a confined space increases risk for personnel involved and may interfere with the procedure. Proximity to a water source, lighting and easy access to emergency supplies is important.

It is imperative to position the patient properly when heavily sedated. Animals maintained in lateral recumbency should have their neck extended at all times to maintain upper airways patency. To allow flow of rumen fluid and saliva out from the oral cavity, pad or towels placed underneath the head and neck junction so that the opening of the mouth is below the level of the larynx. Down forelimb be pulled forward to prevent radial nerve paralysis. A thick pad or towels be placed underneath the down shoulder for further protection. Appropriate dorsal positioning especially in adult cattle can be difficult to obtain in a field setting. Headneeds to be stabilized, rather than hanging to prevent excessive tension on neck structures. Short and thick neck and horn conformation of many cattle and goat breeds can make proper orientation difficult to achieve. Care be taken to protect the eyes in heavily sedated or anesthetized animals especially when placed in lateral recumbency; the lids of the down eye should be closed and protected by a towel or pad.

Once an injectable sedation protocol is selected, the route of administration of these drugs (IV, IM, SC), and the demeanor must be decided by the clinician. Overall, the intravenous route is the most effective method of administration in terms of bioavailability and onset of action. However, the intravenous route may not always be practical under field conditions, especially when dealing with unruly large cattle and swine; in these animals, the intramuscular or subcutaneous route be used initially to achieve sedation. The limitations of intramuscular and subcutaneous injection include incomplete bioavailability, delayed onset of action, and the limited volume that be administered.

All patients sedated or anesthetized with injectable drugs be monitored closely. Heart rate, respiratory rate, mucous membranes colour and capillary refill time checked at regular intervals during and following the anesthetic episode. Also, anesthetic depth should be assessed throughout as described in Table 1.

Table 1. Clinical signs used to monitor anesthetic depth in food animals

Anesthetic depth	Eye position	Palpebral reflex
Light	Central	Present
Adequate	Rolled down (toward nose)	Absent
Deep	Central	Absent

Chemical Restraint Techniques

Xylazine - *α_2-Adrenergic agonist*

Xylazine sedation is useful for facilitating short diagnostic or therapeutic procedures on less cooperative patients. Although patients generally tolerate mildly uncomfortable stimuli, not very reliable for, standing sedation to provide significant analgesia. Duration of xylazine sedation and analgesia is dose dependent, generally lasting about 30 to 40 minutes following intravenous administration in

standing or laterally recumbent patients. In dorsally recumbent patients, the duration of enhanced cooperation provided by intravenous xylazine may be as short as 20 minutes. Duration typically doubled with intramuscular administration, although intensity is commensurately reduced. Clinicians who have tried the "ketamine stun" technique tend to prefer it to pure xylazine chemical restraint (*Table 2*).

Xylazine (0.05 mg/kg IV or 0.1 mg/kg IM) results in recumbency in 50% of tractable cattle. Xylazine (0.1 mg/kg IV or 0.2 mg/kg IM) results in recumbency in most tractable cattle. Anxious or unruly patients are more resistant and somewhat higher doses of xylazine may be required to produce recumbency. Titrated administration (e.g., initial conservative dose that supplemented if necessary) minimizes the amount of xylazine administered and the degree of adverse side effects produced. Physical methods can also be used to produce recumbency once the patient is sufficiently sedated.

Table 2. Dose range of xylazine expected to produce standing sedation with a low incidence of recumbency

Patient type	IV[a]	IM
Dairy breeds	0.0075-0.01 mg/kg	0.015-0.02 mg/kg
Tractable cattle	0.01-0.02 mg/kg	0.02-0.04 mg/kg
Anxious cattle	0.02-0.03 mg/kg	0.04-0.06 mg/kg
Extremely anxious or unruly cattle	0.025-0.05 mg/kg	0.05-0.1 mg/kg

Abbreviations: IM, intramuscularly; IV, intravenously.

[a]Administering the IV dose IM further reduces the possibility of recumbency.

Detomidine

Detomidine is a more potent α_2-adrenergic agonist. Because ruminants have increased sensitivity to xylazine, the dose relationship between xylazine and detomidine in ruminants does not reflect these differences (Table 3). Detomidine doses used in ruminants are similar to those used in equine patients. Detomidine produces greater cardiorespiratory depression than xylazine and not be used in animals to produce recumbent sedation.

Table 3. Dose range of detomidine expected to produce standing sedation with a low incidence of recumbency

Patient type	IV[a]	IM
Tractable cattle	0.002-0.005 mg/kg	0.006-0.01 mg/kg
Anxious cattle	0.005-0.0075 mg/kg	0.01-0.015 mg/kg
Extremely anxious or unruly cattle	0.01-0.015 mg/kg	0.015-0.02 mg/kg

Information regarding the use of detomidine in ruminants is limited. The dose ranges provided are estimates and should be adjusted based on experience.

Abbreviations: IM, intramuscularly; IV, intravenously.

[a]Administering the IV dose IM further reduces the possibility of recumbency.

α_2-Adrenergic agonist and opioids

An opioid is preferred (butorphanol or morphine) be administered to augment the level of systemic analgesia in ruminants when sedated with **α_2-**adrenergic agonists like xylazine or detomidine. Butorphanol (0.05-0.1 mg/kg IV or IM) in smaller ruminants, 0.02-0.05 mg/kg IV or IM in larger ruminants) or morphine (0.05-0.1 mg/kg IV or IM) can be administered with the initial dose of α_2, or added in situations when patient's cooperation needs improvement. The α_2 dose can typically be reduced somewhat when used in conjunction with an opioid.

Ketamine

Ketamine is by far the most common injectable anesthetic agent used in large animal or farm animal practice. Ketamine is an N-methyl-D-aspartate receptor antagonist, possesses potent analgesic effects at sub anesthetic doses. Sub anesthetic doses of ketamine used in chemical restraint in "Ketamine Stun".

Telazol

Telazol is a combination of equal parts by weight of tiletamine hydrochloride a dissociative anesthetic similar to ketamine, a N-methyl-D-aspartate receptor antagonist, and Zolazepam hydrochloride a benzodiazepine with minor tranquilizing properties. Due to high cost of this product, primarily used in large animal practice for capturing intractable patients.

Ketamine Stun

The author prefers ketamine stun in cattle in performing caesarian sections, vasectomy, caudal epididymectomy etc. Ketamine is a dissociative anesthetic commonly used in veterinary medicine. Ketamine possesses potent analgesic effects when administered at subanesthetic doses. Adding a small dose of ketamine to more traditional chemical restraint combinations greatly enhances the level of patient cooperation. This technique is called the **"ketamine stun"** (or stun) because of the stunned effect it produces in patients when administered IV at doses that produce recumbency. These patients appeared to be awake, but seem oblivious to surroundings and procedure performed. The intravenous effect is quite brief (approximately 15 minutes) and patients typically stand and appear fairly normal at that time, this state can be referred to as semi-anesthetized, but perhaps chemical hypnosis is more appropriate. Dosing must be more conservative when using the ketamine stun technique in standing patients. This limits the degree of systemic analgesia relative to what can be achieved in recumbent patients, but still provides improved patient cooperation when compared with more traditional methods of standing chemical restraint in both ruminants and horses.

The $\alpha_2$adrenergic agonist (xylazine) possess potent sedative and analgesic effects. Opioids (butorphanol) are analgesic, but they possess central nervous system effects that when combined with a tranquilizer or sedative produces a greater level of mental depression. Ketamine is an *N*-methyl-D-aspartate receptor antagonist that possesses potent analgesic effects at subanesthetic doses. Ketamine was included in the stun technique for its analgesic properties, likely contributes to the mental aspect of

the enhanced cooperation exhibited by patients under the influence of the ketamine stun technique. By combining drugs, one is able to use smaller doses of the individual components while still achieving the desired level of effect.

Ketamine stun techniques divided into two broad categories: standing and recumbent. The standing ketamine stun used primarily in large ruminants and horses. The recumbent ketamine stun, used primarily in the small ruminants, camelids, and foals. The level of effect achieved is determined by three variables (Dose, Route of administration, Initial demeanor of the patient). The stun cocktail can be administered IV, IM or SQ depending on the systemic analgesia, patient cooperation, and duration desired (Table 4).

Table 4. Route of administration determines the relative impact of the ketamine stun technique

Parameter	Relative Ranking
Intensity (analgesia/cooperation)	IV >> IM > SQ
Onset	IV >> IM > SQ
Duration of effect	SQ > IM >> IV

Clinical application of the ketamine stun in food animal patients can be divided into four basic categories.

Intravenous recumbent stun

The intravenous recumbent stun used for short procedures or procedures requiring high level of systemic analgesia and patient cooperation.

A combination of xylazine (0.025-0.5 mg/kg), butorphanol (0.05-0.1 mg/kg), and ketamine (0.3-0.5 mg/kg) is administered IV. Onset is approximately 1 minute. Patients gracefully become recumbent. Patients seem to be awake, but seem oblivious to surroundings and procedures being performed. Mild random head or limb motion is not unusual, but purposeful movement or vocalization are signs of an inadequate stun level and additional drug should be administered. One half of the initial ketamine dose should be administered IV and is often effective. If, after allowing 60 to 90 seconds for onset, this additional half dose of ketamine fails to produce the desired level of analgesia, a second half dose of ketamine along with one half of the initial dose of xylazine should be administered IV.

The level of systemic analgesia produced varies depending on the doses administered, but tends to be intense. Surgical levels of analgesia isachieved with this technique, but the use of local anesthetic blockade should be used whenever feasible to reduce the risk of patient awareness and stress. Duration of the stun effect is approximately 15 minutes and patients typically are able to stand and walk immediately or shortly after this point. The intravenous recumbent stun is designed for short procedures. One should plan ahead and work fast. Supplemental doses of ketamine or xylazine can be administered to extend duration, but this technique is not intended for procedures that are expected to last significantly beyond the 15-25

minute range. The degree of extension is relative to the amount of supplemental drug administered.

The recumbent intravenous stun has proved very useful for facilitating a wide variety of short procedures in camelids and small ruminants.

Intramuscular or subcutaneous recumbent stun

The intramuscular or subcutaneous recumbent stun used for procedures requiring a longer duration of chemical restraint. The level of systemic analgesia is not as intense, and local anesthetic blockade should be used to reduce the risk of patient awareness and stress. Umbilical hernia repair is an example of the procedures performed using this technique. This approach is also useful for improving cooperation in patients that have gone down before or during a surgical procedure.

A combination of butorphanol (0.025 mg/kg), xylazine (0.05 mg/kg), and ketamine (0.1 mg/kg) is administered IM or SQ. Subcutaneous administration is preferred because it provides a slightly longer duration of effect. Onset time is approximately 3 to 10 minutes. Patients are obtunded enough to require (and tolerate) intubation when placed in dorsal recumbency. The duration of effect with subcutaneous administration is approximately 45 minutes. Patients should be ambulatory within 30 minutes following this point.

The level of systemic analgesia produced by the intramuscular or subcutaneous recumbent stun is not as intense, but this approach does provide an enhanced level of patient cooperation that can make procedures much more pleasant for both patient and clinician.

Intravenous standing stun

The intravenous standing stun typically used to provide a transient improvement in patient cooperation. Small doses of intravenous ketamine can markedly improve the degree of patient cooperation in standing chemical restraint. Butorphanol (0.05-0.1 mg/kg IV or IM in smaller ruminants, 0.02-0.05 mg/kg IV or IM in larger ruminants) or morphine (0.05-0.1 mg/kg IM or IM) be added to augment the level of analgesia and patient control.

Intramuscular or subcutaneous standing stun

5-10-20 technique

The intramuscular or subcutaneous standing stun used for most standing procedures in ruminant patients. The level of systemic analgesia is limited and local anesthetic blockade be used to reduce the risk of patient awareness and stress. Standing flank laparotomy is an example of the procedure performed using this technique.

A combination of butorphanol (0.01 mg/kg), xylazine (0.02 mg/kg), and ketamine (0.04 mg/kg) is administered IM or SQ. In a 500 kg cow this equates to butorphanol (5 mg), xylazine (10 mg), and ketamine (20 mg). For a 680 kg patient the doses are 7 mg butorphanol, 15 mg xylazine, and 25 mg ketamine. Morphine (25 mg for 500 kg cow, 30 mg for 680 kg cow) can be substituted for butorphanol.

Subcutaneous administration is preferred to minimize the risk of recumbency. In very unruly cows, intramuscular administration provides better patient control. Onset is 5 to 10 minutes with subcutaneous administration. Cows stood quietly during the caesarean sections (many were ill mannered before the ketamine stun). The duration of effect is approximately 60-90 minutes. Additional xylazine and ketamine can be administered SQ to extend the duration of chemical restraint. Recumbency has occasionally occurred with re-administration of 50 of all three components. Current recommendation for supplemental drug administration is 25%-50% of the initial xylazine and ketamine doses (0-2.5-5) and (0-5-10), respectively, depending on the degree of cooperation and time required to complete the procedure.A similar approach (10-20-40 technique) has been used successfully in adult bulls. Preputial surgery (with local anesthetic block) is an example of the procedures performed using this technique.

Anesthetic Drugs (Table 5) & Anesthetic Protocols (Table 7 & 8)

Table 5. Indications, dosage and side effects of common anesthetic drugs used in food animals

Anesthetic drug	Drug class	Indications	Side effects to consider	Dosage (mg/kg)	Route	Duration (min)
Xylazine	α-2 Agonist	Short-term sedation Muscle relaxant Mild analgesia	Cardiorespiratory depression Bloat, recumbency Hyperglycemia Abortion (3rd trimester)	0.05 0.1-0.2	IV IM	20-30 30-40
Detomidine	α-2 Agonist	Longer sedation 20xanalgesicsedative than xylazine	Similar to Xylazine except: Safe to use in pregnant cows ↓ Likelihood of recumbency	0.005-0.02 0.02-0.04	IV IM	Dose dependent
Ketamine	Dissociative	In association for general anesthesia	Respiratory depression No muscle relaxation	2-3 3-4	IV IM	15-20 20-30
Diazepam	Benzo diazepine	Anticonvulsant Sedation	Cardiorespiratory depression	0.5-1: seizure 0.05-0.2: sedation	IV slow IV slow	30-45
Butorphanol	Opioid agonist/ antagonist	Analgesia Sedation	May induce excitation if given by itself	0.05-0.2 0.2-0.5	IV IM	45-60
Morphine	Pure opioid agonist	Analgesia	Respiratory depression ↓ GI motility	0.05-0.2 0.05-0.5	IV, IM IM, SC	240-360 (4-6 hrs.)
Guaifenesin	Central Skeletal Muscle Relaxant	Muscle relaxation	No analgesia	100	IV drip	Drip rate dependent
Tiletamine/ Zolazepam	Dissociative /Benzodiazepine	General anesthesia Analgesia Muscle relaxation	Respiratory depression Long but smooth recovery	5	IM	60-90
Acepromazine	Dopamine & α-1 antagonist	Mild sedation Calming effect	No analgesia, hypotension Penile prolapse → trauma	0.01-0.02 0.03-0.1	IV IM	120-240 (2-4 hrs.)

Table 6. Dosage of common reversal anesthetic drugs used in food animals

Anesthetic drug	Drug class	Indications	Side effects to consider	Bo/OV/Cap/ Camelids Dosage (mg/kg)	SwineDosage (mg/kg)	Route
Tolazoline	α-2 Antagonist	Reversal α-2 agonist	Adverse reactionpossible when given IV fast	1-2	1-2	IV slow IM, SC
Yohimbine	α-2 Antagonist	Reversal α-2 agonist		0.125-0.2	0.1-0.2	IV slow, IM
Atipamazole	α-2 Antagonist	Reversal α-2 agonist	Most appropriate α- 2 reversal in camelids	0.125-0.2	0.2	IM, SC
Flumazenil	Benzodiazepine antagonist	Reversal for benzodiazepines		0.1	0.01	IV
Naloxone	Opioid antagonist	Reversal for opioids		0.03	0.5-2	IV, IM

Table 7. Injectable anesthetic protocols for bovine, ovine and caprine species

Protocol	Dosage	Route	Species	Indications	Duration (min)
Telazol 500 mg Ketamine 250-400mg Xylazine 100 mg	1.25-1.5 mL/100 Lbs. (Ov, Cap) 1 mL/100 lb (Bov)	Pole syringe or dart gun → IM	Bov Ov Cap	Capture & immobilization	~40-60
*Butorphanol *Xylazine *Ketamine	0.025 mg/kg 0.05 mg/kg 0.1 mg/kg	IV IM or SC	Bov	Standing sedations (bucking stock) Recumbent sedation (Routine surgery)	~15-20 ~30-40
*Butorphanol *Xylazine *Ketamine	0.05-0.1 mg/kg 0.025-0.05 mg/kg 0.3-0.5 mg/kg	IV	Bov	Short procedure requiring lateral or sternal recumbency, analgesia, and patient cooperation	
*Butorphanol *Xylazine *Ketamine	5 mg or 10 mg 10 mg or 20 mg 20 mg or 40 mg	IM or SC	Bov	Chemical restraint for standing C-section in beef cows (340 - 660 Kg Body weight)	~60-90
5%Guaifenesin 1L Ketamine 1000mg Xylazine 50-100mg *"IV triple drip"*	Induction: 1mL/kg Maintenance: 2 mL/kg/hr.	IV -> catheter (drip set)	Bov	Procedure requiring good muscle relaxation (ex: *cast application*)	~60-90
Xylazine (X) Ketamine (K)	(X): 0.05 mg/kg (K): 2 mg/kg (X): 0.1 mg/kg (K): 4mg/kg	IV IM	Bov Ov Cap	General anesthesia – routine surgery Prolongation: administer ½ of initial ketamine dose	~30-40 ~40-60

Ketamine stun

Table 8. Injectable anesthetic protocols for swine

Protocol	Dosage	Route	Indications	Duration (min)
Acepromazine	0.2-0.5 mg/kg	IM	Sedation	~ 30
Xylazine	0.5-3 mg/kg	IM	Sedation	~ 30
Medetomidine Butorphanol Midazolam	10-20 mg/kg 0.1-0.2 mg/kg 0.1-0.5 mg/kg	IM	Sedation	~ 60
Xylazine Glycopyrrolate Butorphanol	1 mg/kg 0.01 mg/kg 0.05 mg/kg	IM	Sedation in pot belly pigs (especially geriatric patients)	~ 60
Xylazine Midazolam Ketamine	1 mg/kg 0.2 mg/kg 2-10 mg/kg	IM	Sedation for caesarian section (use higher ketamine dosage for heavier sedation)	~ 60
Midazolam Ketamine	0.5 mg/kg 5-10 mg/kg	IM	Sedation for geriatric pot belly pigs.	~ 30-40
Pig cocktail #1: 5 ml Telazol 500 mg - powder Ketamine 250 mg Xylazine 250 mg	1 mL/50kg	IM	General anesthesia (prolonged recovery)	~ 60-90
Pig cocktail #2: 5 ml Telazol 500 mg - powder Xylazine 300 mg Sterile water 2 mL	1 mL/25kg	IM	General anesthesia (less chance of apnea compared to pig cocktail #1) Inguinal hernia Castration	~ 60
Pig cocktail #3: 5 ml Telazol 500 mg - powder Xylazine 150 mg Sterile water 3.5mL	4 mL/200 kg	IM	General anesthesia Cesarean section Hernia repair	~60

***Xylazine 1 mL = 100 mg**

Withdrawal Times Suggested

Since most anesthetic drugs have a short half-life and are typically administered once at a low dose on an mg/kg basis, the incidence of volatile residues in meat or milk is fairly rare. In addition, anesthetized food animals going through a surgery are unlikely to be slaughtered shortly after the procedure. The time necessary for recovery and healing of the surgical wound is usually long enough for most anesthetic drugs to clear before slaughter. It is recognized that tests for anesthetic residue are not performed routinely, in contrast to tests for antibiotic residues. Reaction in people caused by consumption of milk or meat contaminated with anesthetic drugs residues has not been documented.

Table 9. Recommended withdrawal times associated with injectable anesthetic drugs (*Bovine*)

Anesthetic drug	Meat withdrawal (days)	Milk withdrawal (hours)
Xylazine	4	24
Detomidine	3	72
Acepromazine	7	48
Ketamine	3	72
Thiopental	4	Not determined (ND)
Tiletamine/Zolazepam	30	ND
Diazepam	30	ND
Midazolam	14	ND
Butorphanol	19	72
Guaifenesin	3	48
Atropine	14	72
Tolazoline	8	48
Yohimbine	7	72

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Full Length Articles

Methane production potential of feed ingredients estimated by *in vitro* gas production test

M.Ramachandran, A. Bharathidhasan and V.Balakrishnan
Tamil Nadu Veterinary and Animal Sciences University
Department of Animal Nutrition, Madras Veterinary College,
Vepery, Chennai – 600 007, India.

ABSTRACT

This study was conducted to investigate methane production potential of feed ingredients to develop a database on methane production. Feed ingredients such as cereal grains, cereal by-products and protein supplements were tested for methane production potential using *in vitro* gas production technique. *In vitro* true digestibility (IVTD) of cereal grains ranged from 60.1 to 96.7% and oats grain (76.2%) and distiller's grain (60.1%) had lower (P<0.05) values than other cereal grains. Among the cereal by-products, wheat bran showed highest (P<0.05) IVTD (74.9%) than rice bran (42.7%). IVTD of cottonseed oil cake, black gram and sunflower oil cake was lower (P<0.05) than other protein supplements. Methane production potential of cereal grains at half life (t1/2) ranged from 0.66 to 2.85 ml/100 mg truly digested substrate and the difference was significant (P<0.05), however, maize grain, sorghum grain, bajra and broken rice did not vary among themselves. Average methane production potential of cereal by-products at half life (t1/2) and 24 hrs was 1.27 and 1.81 ml/100 mg truly digested substrate, respectively. Average methane production potential of protein supplements at half life (t1/2) and 24 hrs was 1.39 and 1.75ml/100 mg of truly digested substrate, respectively and the difference was statistically significant (P<0.05). Maximum (P<0.05) methane production potential at half life (t1/2) was recorded for black gram (4.07 ml/100 mg truly digested substrate). Lowest methane production potential both at half life (t1/2) and 24 hrs were recorded in fish meal and spirulina. It can be concluded that among cereal grains, methane production potential was higher (P<0.05) in oats grain at half life (t1/2) and all the cereal grains had similar methane production potential at 24 hrs. Among cereal by-products, wheat bran had higher (P<0.05) methane production potential both at half life (t1/2) and 24 hrs. Among protein supplements, black gram had significantly (P<0.05) higher methane production potential at half life (t1/2) and horse gram had significantly (P<0.05) higher methane production potential at 24 hrs.

Key Words: Methane, database*, in vitro* true digestibility

*Corresponding author: Professor and Head, Livestock Farm Complex, Veterinary College and Research Institute, TANUVAS, Orathanadu, Thanjavur – 614 625, India

INTRODUCTION

Methane is second major gas after carbon dioxide responsible for the warming of environment and ozone layer depletion. It is a potent green house gas as it has 23 times higher global warming potential than carbon dioxide (IPCC, 1996). Estimates of global methane production ranged between 350-820 Tg/year (Khan *et al*., 2001). Ruminants contribute about 30% of the world total methane production. Global warming and ozone layer depletion due to increased emission of green house gases in the atmosphere have drawn worldwide attention with an alarming stage of iceberg melting, increased ocean level, local and global eco-system upsets, changes in the rainfall patterns, changes in pathogenesis of plants, animals and human beings and alteration in life of the people (Kumar *et al.,* 2008). Several reports of the United Nations inter-governmental panels on climate changes indicated the urgency of the problem. IPCC (2001) has warned that by the mid of this century the globe's temperature will rise just like anything up to 5.8^{o}C.

Livestock are one amongst the largest single source of methane emission with 80–115 million tonnes per year, equivalent to 15–20% of total anthropogenic methane (IPCC, 2001). Ruminal microorganisms are responsible for the emission of methane from livestock (cattle, buffalo, sheep, goats, camel, *etc.*). The global cattle population is responsible for 73% of methane emissions of all livestock (McCrabb and Hunter, 1999). Tropical grasses are of low to moderate digestibility (on average 13% lower dry matter (DM) digestibility than temperate grasses) and are often deficient in critical nutrients such as protein and phosphorus. Under such conditions, methane produced during ruminal fermentation represents a loss of 10–11% of gross energy intake. The enteric methane contributes approximately 30–40 per cent of total methane produced from agricultural sources (Moss *et al*., 2000). Methane from enteric fermentation by ruminants is not only an important greenhouse gas associated with environmental problems, but it also represents a loss of feed energy (20–150 kJ/MJ) intakes (Singh *et al*., 2005). Therefore, developing feeding strategies to minimize methane emission is desirable in long-term mitigation of emission of greenhouse gases into the atmosphere and for short-term economic benefits.

This study was conducted to investigate *in vitro* methane production potential of different feed ingredients to develop a database on methane production to estimate the methane emission from ruminant livestock precisely and to develop methane mitigation strategies to reduce global warming and enhance the efficiency of nutrient utilization.

MATERIALS AND METHODS

Collection, processing and chemical analysis of feed ingredients

Feed ingredients such as cereal grains, cereal by-products and protein supplements were collected from Tamil Nadu and these samples were dried in a hot air oven at about 50-60^{0}C and ground using 1 mm sieve. Total ash (TA) and ether extract (EE) content were estimated as per the procedure of AOAC, (1995). Organic matter (OM)

was calculated based on the total ash (TA) content. Neutral detergent fibre (NDF) content was analyzed as per the procedure of Goering and Van Soest, (1970). The OM, EE and NDF content of different feed ingredients is given in Table 1.

Table 1: Organic matter (OM), Ether extract (EE) and Neutral detergent fibre (NDF) content of feed ingredients (% Dry matter basis) (Mean ±SE)

Feed ingredient	OM (%)	EE (%)	NDF (%)
Cereal grains			
Maize	98.2±0.15	5.06±0.35	14.5±1.35
Sorghum	98.2±0.13	3.75±0.41	14.4±0.71
Ragi	97.0±0.10	1.10±0.06	17.0±0.99
Bajra	96.5±0.12	4.16±1.50	11.1±1.89
Oats	96.7±0.05	3.55±0.13	30.8±0.75
Broken rice	94.8±0.65	1.43±0.01	42.3±1.44
Distiller's grain	94.6±0.03	1.90±0.02	49.8±0.51
Cereal by-products			
Rice bran	85.0± 1.51	4.22±0.81	68.7±3.81
Wheat bran	91.5±0.89	2.67±0.65	45.3±2.35
Protein supplements			
Groundnut oil cake	92.4±1.05	6.72±0.46	17.6±3.25
Coconut oil cake	93.6±0.35	12.6±0.80	35.5±1.89
Soybean meal	92.6±0.74	1.40±0.25	20.5±2.67
Cottonseed oil cake	95.2±0.20	9.91±0.31	45.9±3.32
Sunflower oil cake	91.0±0.37	0.96±0.04	50.4±2.34
Gingely oil cake	93.1±0.22	1.22±0.08	14.2±1.10
Linseed	97.7±0.01	43.5±0.07	22.6±0.94
Horse gram	95.9±0.01	0.94±0.01	53.8±0.50
Fish meal	58.9±0.01	6.08±0.14	16.5±0.40
Green gram	94.7±0.01	1.32±0.04	31.0±2.77
Black gram	95.6±0.09	1.68±0.03	46.2±1.22
Spirulina	92.8±0.06	1.13±0.04	1.68±0.06

In vitro gas production

Collection of rumen inoculum

Rumen content was collected from male calves fed on paddy straw based rations using stomach tube and strained through 4 layers of muslin cloth. The strained rumen liquor (SRL) was transported to the laboratory in a cud transport container having the facility for CO_2 flushing and temperature maintenance.

In vitro gas production test

Five media solutions were prepared individually and were mixed later as specified by Menke and Steingass, (1988). Total volume of buffer required was calculated based on the number of samples incubated. The required quantity of water, micro, macro, buffer and resazurin were mixed in a flat bottom flask and kept in the incubator at about 39°C.

Ground samples (1mm) of about 200 mg were weighed and transferred carefully in to the 100 ml calibrated glass syringes. After weighing all the samples, vaseline was applied to the piston and inserted in to the syringes. The nozzles of the syringes were closed with rubber cork. The syringes were kept in an incubator at 39°C a day before the incubation.

The required volume of strained rumen liquor was measured and added to the medium in the flask. Carbon dioxide was flushed through the medium. Exactly 30 ml of rumen inoculum was dispensed into the syringes through silicone tube fitted to the nozzle. After removing the silicone tube the nozzle was closed using a rubber cork after removing the gas bubbles. Then the syringes were incubated in a water bath maintained at 39°C in triplicates. The gas production was measured at 2, 4, 6, 8, 12, 24, 36, 48, 72 and 96 hours interval and corrected with blank. The gas production at different intervals was analysed using Graph Pad Prism (version 5.0) to estimate half life (t1/2).

Estimation of *in vitro* true digestibility (IVTD)

Ground samples (1mm) of about 500 mg were weighed and transferred carefully in to the 100 ml calibrated glass syringes. Media solution for the estimation of *in vitro* true digestibility was prepared as per the procedure of Makkar *et al*. (1995). Exactly 40 ml of rumen inoculum with double strength buffer was dispensed into the syringes through silicone tube fitted to the nozzle. After removing the silicone tube the nozzle was closed using a rubber cork after removing the gas bubbles. Then the syringes were incubated in a water bath maintained at 39°C in triplicates. The experiments for the estimation of *in vitro* true digestibility and methane emission were carried out simultaneously.

After recording total gas production, *in vitro* true digestibility was estimated at 24 hours of incubation. After the end of incubation, the contents were carefully transferred into spoutless beaker by repeated washing with neutral detergent solution (NDS). The contents were refluxed for one hour using Fibertec equipment and filtered through pre-weighed Gooch crucible (Grade I). The residues were dried in the hot air oven and weighed.

$$\text{True digestibility (\%)} = \frac{\text{DM of feed taken for incubation - NDF residue}}{\text{DM of feed taken for incubation}} \times 100$$

Estimation of methane emission

Ground samples (1mm) of about 200 mg were weighed and transferred carefully in to the 100 ml calibrated glass syringes. Exactly 30 ml of rumen inoculum was dispensed into the syringes through silicone tube and the nozzle was closed using a rubber cork after removing the gas bubbles. Then the syringes were incubated in a water bath maintained at 39°C in triplicates and the experiment was repeated on 3 different days. Total gas production was recorded both at half life and 24 hours for feedstuffs with less than 16 hours half life. For other feed ingredients gas samples were collected at half life after recording the total gas production. Gas samples were collected in vacuum container to estimate the concentration of methane using Gas Chromatography.

Estimation of methane concentration using Gas Chromatograph

Methane concentration in different gas samples collected during the *in vitro* studies was estimated using Gas Chromatograph (Claurus 500, Perkin Elmer) fitted with Flame Ionization Detector (FID) and capillary column (30 meter length and 250 micrometer dia) using a calibration gas consisting of 22.53% methane, 1.05% hydrogen, 33.30% carbon dioxide and 43.12% nitrogen. Helium was used as carrier gas with oven temperature at 60° C, injector temperature at 100°C and detector temperature at 200°C.

Based on the true digestibility, methane production potential per 100 mg truly digested substrate was calculated for all the feed ingredients.

Statistical analysis

All the *in vitro* experiments adopted a completely randomized design (CRD). The methane production potential was statistically analyzed using one way analysis of variance (One Way - ANOVA) to compare the means as per the procedure of statistical analysis system (SAS/ SPSS version 15.0 for windows). When significant difference was detected the multiple range test was used to separate the mean value.

RESULTS AND DISCUSSION

The results of *in vitro* true digestibility (IVTD), half life and methane production potential of feed ingredients is given in Table 2. Results revealed that IVTD of cereal grains ranged from 60.1 to 96.7% and oats grain (76.2%) and distiller's grain (60.1%) had lower ($P<0.05$) values than other cereal grains. Lower ($P<0.05$) IVTD in oats grain and distiller's grain might be due to the higher content of structural carbohydrate (NDF) (Table 1) which is not easily available for microbial digestion. High digestibility of maize, sorghum, ragi, bajra and rice is attributed to high content of easily digestible carbohydrate. Similar IVTD for maize and sorghum grains were also reported by Hervas *et al.,* (2004). Cereal by-products comparatively had lower ($P<0.05$) IVTD than cereal grains

because of higher cell wall carbohydrate (NDF) (Table 1).

Results of IVTD of protein supplements indicated that significant difference (P<0.05) was found among various protein supplements. Groundnut oil cake, soybean meal, gingely oil cake, linseed, fishmeal, horse gram and spirulina had similar IVTD values. *In vitro* true digestibility of cottonseed oil cake, black gram and sunflower oil cake was lower (P<0.05) than other protein supplements which may be attributed to high cell wall content (Table 2).

Table 2: *In vitro* true digestibility (%), half life (hr) and methane production potential (ml) of feed ingredients

Name of the feed ingredient	*In vitro* true digestibility (IVTD) (%)	Half life ($t_{1/2}$) (hr)	Methane production potential (ml/100 mg truly digested substrate)	
			Half life ($t_{1/2}$)	24 hrs
Cereal grains				
Maize	89.4 ± 0.30^{cd}	12.2	0.95 ± 0.13^{ab}	1.75 ± 0.33^{a}
Sorghum	96.7 ± 0.54^{d}	9.47	0.82 ± 0.14^{a}	2.31 ± 0.72^{a}
Ragi	87.1 ± 0.39^{c}	15.6	0.66 ± 0.14^{a}	1.59 ± 0.10^{a}
Bajra	87.5 ± 0.50^{cd}	13.4	1.62 ± 0.12^{abc}	3.71 ± 0.24^{a}
Oats	76.2 ± 1.02^{b}	14.9	2.85 ± 0.66^{c}	3.77 ± 0.76^{a}
Broken rice	88.2 ± 0.27^{cd}	12.6	1.72 ± 0.13^{abc}	2.10 ± 0.40^{a}
Distiller's grain	60.1 ± 1.75^{a}	14.4	2.41 ± 0.19^{bc}	3.78 ± 0.47^{a}
Average	**83.6**	**13.2**	**1.58**	**2.72**
Cereal by-products				
Rice bran	42.7 ± 0.63^{a}	10.4	0.55 ± 0.13^{a}	0.91 ± 0.33^{a}
Wheat bran	74.9 ± 0.93^{b}	12.4	1.98 ± 0.32^{b}	2.71 ± 0.46^{b}
Average	**58.8**	**11.4**	**1.27**	**1.81**
Protein supplements				
Groundnut oil cake	93.8 ± 0.58^{ef}	5.81	1.05 ± 0.05^{abc}	2.00 ± 0.08^{bcd}
Coconut oil cake	84.0 ± 0.99^{d}	7.52	1.38 ± 0.18^{abc}	2.47 ± 0.17^{cd}
Soybean meal	95.0 ± 0.05^{f}	6.51	0.96 ± 0.09^{abc}	1.70 ± 0.09^{abc}
Cottonseed oil cake	45.9 ± 0.82^{a}	24.0	0.75 ± 0.13^{abc}	-
Sunflower oil cake	70.4 ± 1.14^{c}	10.6	0.72 ± 0.21^{abc}	1.28 ± 0.23^{abc}
Gingely oil cake	95.5 ± 0.25^{f}	6.99	1.18 ± 0.17^{abc}	2.01 ± 0.20^{bcd}
Linseed	87.4 ± 0.42^{de}	17.6	1.35 ± 0.06^{abc}	-
Horse gram	90.4 ± 0.21^{def}	14.2	1.66 ± 0.57^{bcd}	3.13 ± 0.64^{e}
Fish meal	94.6 ± 0.66^{f}	11.3	0.40 ± 0.27^{a}	0.62 ± 0.33^{a}
Green gram	85.2 ± 3.18^{d}	19.0	2.54 ± 0.18^{d}	-
Black gram	54.0 ± 3.29^{b}	33.2	4.07 ± 0.07^{e}	-
Spirulina	96.2 ± 0.37^{f}	9.38	0.59 ± 0.07^{ab}	0.77 ± 0.10^{ab}
Average	**82.7**	**13.8**	**1.39**	**1.75**

^{abcde} Means with different superscripts in a column with respect to cereal grains/cereal by-products/ protein supplements differ significantly (P<0.05).

Methane production potential of cereal grains at half life (t1/2) ranged from 0.66 to 2.85 ml/100 mg truly digested substrate and the difference was significant (P<0.05). However, maize grain, sorghum grain, bajra and broken rice did not vary among themselves. Oats grain produced maximum methane at half life (t1/2) (2.85 ml/100 mg truly digested substrate) compared to all other cereal grains which may be due to high NDF (30.8%) and low digestibility (76.2%). Lowest methane was produced by ragi grain at 24 hrs (1.59 ml/100 mg truly digested substrate) and highest methane was produced by bajra grain, oats grain and distiller's grain at 24 hrs (3.71, 3.77 and 3.78 ml/100 mg truly digested substrate respectively), however, there was no significant difference found among the cereal grains. Average methane production potential of cereal grains both at half life (t1/2) and 24 hrs was 1.58 and 2.72 ml/100 mg truly digested substrate, respectively.

High methane production of cereal grains compared to cereal by-products and protein supplements might be attributed to high contents of easily fermentable starch, sugars or hemicellulose as substrate to rumen microbes for fermentation. Cereal grains contain high amount of NFE which is readily fermented by ruminal microbes and provide the large amount of substrates to microbes for methane production. Besides the high amount of easily fermentable substrates, Bonhomme, (1990) reported that grains rich in soluble carbohydrates increase the population of cilliate protozoa and stimulate their hydrogen transfer to methanogens resulting in high methane production. Lee *et al*. (2003) reported that the methane production potential of corn at 6 and 24 hrs was 4.03 and 10.33 ml/0.2g DM, respectively. Methane production potential of oat grain at 6 and 24 hrs was 4.34 and 6.87 ml/0.2 g DM, respectively (Lee *et al*., 2003).

Methane production potential both at half life (t1/2) and 24 hrs were maximum (P<0.05) in wheat bran (1.98 and 2.71 ml/100 mg truly digested substrate, respectively). Similarly, Lee *et al*. (2003) reported that methane production potential of rice bran was lower than wheat bran. Average methane production potential of cereal by-products at half life (t1/2) and 24 hrs was 1.27 and 1.81 ml/100 mg truly digested substrate, respectively. Rice bran contains high concentration of unsaturated fatty acid. Czerkawski *et al*. (1966) reported that unsaturated fatty acids are hydrogenated by rumen microbes resulting in low pressure of hydrogen which is pre-requisite for reduction in methane production. In addition, fat, itself, is considered to inhibit methane production by stimulating propionate production and inhibiting the protozoa activity as well as inhibitory effects on cellulolytic bacteria and feed digestion in rumen.

Average methane production potential of protein supplements at half life (t1/2) and 24 hrs were 1.39 and 1.75 ml/100 mg truly digested substrate, respectively and the difference was significant (P<0.05). Maximum (P<0.05) methane production

potential at half life (t1/2) was recorded in black gram (4.07 ml/100 mg truly digested substrate). Lowest methane production potential both at half life (t1/2) and 24 hrs were recorded in fish meal and spirulina. Lower methane production potential of protein supplements compared to cereal grains might be due to high crude protein generally more than 20% and low amount of fibre. Protein is degraded to ammonia in rumen and it combines to carbon dioxide resulting in production of ammonium carbonate (Getachew *et al*., 1998) resulting in its lower methane production.

It can be concluded that among cereal grains, methane production potential was higher ($P<0.05$) in oats grain at half life (t1/2) and all the cereal grains had similar methane production potential at 24 hrs. Among cereal by-products, wheat bran had higher ($P<0.05$) methane production potential both at half life (t1/2) and 24 hrs. Among protein supplements, black gram had significantly ($P<0.05$) higher methane production potential at half life (t1/2) and horse gram had significantly ($P<0.05$) higher methane production potential at 24 hrs.

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Effect of accelerated feeding in the growth performance and carcass quality in native kids

T.Muthuramalingam[1], E. Rachel Jemimah[2], P.Tensingh Gnanaraj[3], P.Pothiappan[4] and A. Shanmuga sundaram[5]

Tamil Nadu Veterinary and Animal Sciences University
University Research Farm,
Madhavaram Milk Colony, Chennai - 600 051, India

ABSTRACT

A trial was conducted to evaluate the effect of accelerated feeding method in the growth and carcass studies of native goat kids. Thirty male country goat (non – descriptive) kids at the age of 30 - 45 days were selected for this study. The kids were divided into two groups, control and treatment groups, each consist of 15 kids. The control group kids were fed with concentrate feed consisting of 15% crude Protein (CP), 75% Total Digestible Nutrient (TDN), CO4 grass as a sole green fodder and sorghum stover, bengal gram and groundnut tops as a dry fodder. The treatment group kids were fed with concentrate feed containing 21% crude Protein (CP), 75% Total Digestible Nutrient (TDN), C_O4 grass and C_OFS 29 grass as a green fodder and sorghum stover, bengal gram and groundnut tops as a dry fodder. In addition the treatment group kids were fed with supplements such as TANUVAS mineral mixture, probiotics, baking soda and Groviplex®, Ostovet®, Brotone®. The study was conducted for a period of 6 months. The body weight of kids was recorded at fortnight intervals. Parameters such as average feed intake per goat, average total body weight gain, average daily body weight gain and cost of production per kg live weight gain were studied.

The kids were slaughtered at the end of study period and carcass parameters like pre slaughter weight, carcass weight, dressed weight and weights of blood, head, feet, stomach with contents, lungs, heart, kidney, spleen, liver and skin were studied. After analysis of data, significant ($P< 0.01$) difference was noticed between control group and treatment group in terms of final body weight (C -13.28±0.10 kg, T - 17.00±0.06 kg), average total body weight gain (C -6.74 ±0.09 kg, T - 9.98±0.10 kg), average daily body weight gain ($P< 0.05$) (C - 0.04±0.08 kg, T - 0.06±0.09 kg) and cost of production per kg live weight gain (C – Rs.98.15±0.15, T – Rs.72.48±0.12) . There was also highly significant difference ($P < 0.01$) was noticed in carcass quality in terms of pre slaughter weight (C -13.28±0.10 kg,

[1*] Assistant Professor and Corresponding author -
[2] Graduate Assistant, [3]Professor and Head, [5] Assistant Professor, University Research Farm, Madhavaram Milk Colony, Chennai – 600 051.
[4] Assistant Professor, PLAFFS, Madhavaram Milk Colony, Chennai – 600 051.

T - 17.00±0.06 kg), carcass weight (C - 6.25±0.10kg, T - 8.00±0.02 kg), dressed weight (C -5.70±0.15 kg, T - 7.55±0.14 kg), dressing percentage (C - 42.22±0.13%, T - 47.06±0.12%), head (C - 1.13±0.22 kg, T - 1.25±0.02 kg) and stomach (C - 5.10±0.26 kg, T - 6.35±0.2kg). Thus it is concluded that, accelerated feeding significantly improves the body weight gain and carcass yield in native goat kids with low production cost per kg live weight gain.

Key Words: Accelerated feeding, native kids slaughter studies.

INTRODUCTION

Goats are important species of livestock in India. They contribute greatly to the agrarian economy, especially in areas where crop and dairy farming are not economical, and play an important role in the livelihood of a large proportion of small and marginal farmers and landless labourers (Meenakshi Sundaram *et al.,* 2012**).** Their contribution to economy through production of milk, meat, fiber, skin and manure etc., are substantial constituting above 5.4% of GNP of agricultural sector (Sivakumar., 2013). According to FAO (2004), goat contributed about 475 MT of meat worth Rs.4,750 crores to the Indian economy. The demand for goat meat is progressively increasing as Indian consumers prefer goat meat among all and there is no taboo against consumption of chevon. The number of goats available for slaughter is comparatively higher in India; however, the meat yield per animal is lower than the world average as with 11% of the world livestock it only contributes 2.13% of the total meat produced (Sivakumar., 2013). Therefore, it is important to enhance the growth and carcass yield of goats through valuable interventions. Accelerated feeding is one of the interventions to improve the growth and carcass yield. 104, 106 and 117 g/d of average daily gain were observed in goats which were fed with diets containing 11.2, 12.7 and 15.1% of CP, respectively (Lu and Potchoiba., 1990). With this background the current study was formulated to test the hypothesis that increasing the crude protein level in the diet of country goats will improve the growth and carcass quality of kids.

MATERIALS AND METHODS

Experimental design

Thirty numbers of early weaned male native kids (non – descriptive) at the age of 30-45 days were divided into two group viz., control group and treatment group (Accelerated feeding). Each group consists of 15 kids. Duration of the study was six months.

Feed formulation

A ration was formulated for control and treatment group under study as given in table 1.

Table 1.

Ingredients (%)	Ration for treatment group of kids (Accelerated Feeding group)	Ration for control group of kids (Conventional feeding group)
Maize	46.4	15
Soya bean Meal	44.5	-
Dry fish	5	-
DCP	0.7	-
Salt	1.5	0.5
Oil cake	1.8	10
Allzyme	0.1	
Pulses	-	37
Wheat bran	-	35
Mineral mixture	-	2.5
	100	100

Housing management

All the kids both control and treatment group were reared under intensive system where the kids are allowed in a run space during day time and confined in a wooden slatted floor house during night.

Feeding management

All the kids were fed with cow milk (diluted with water at 1:1 ratio boiled and cooled) at the dose of 250-750 ml per kid per day depending upon their body weight till the age of 75 days. All the kids were given access to hygienic ad libitum water throughout the day through automatic waterer.

Control group

The control group kids were fed with concentrate feed consisting of 15% crude Protein (CP), 75% Total Digestible Nutrient (TDN), C_O4 grass as a sole green fodder and and sorghum stover, bengal gram and groundnut tops as a dry fodder.

Treatment group

The accelerated feeding group kid were fed with concentrate feed containing 21% crude Protein (CP), 75% Total Digestible Nutrient (TDN), CO4 grass and COFS 29 grass as a green fodder and sorghum stover, bengal gram and groundnut tops as a dry fodder. In addition, the accelerated feeding group kids were fed with TANUVAS

mineral mixture @ rate of 10 g/day /kid; baking Soda at the rate of 3g/day/kid to prevent bloat; probiotics at the rate of 5 g/ day/kid (Each gram contains *Streptococcus faecalis* T -110 ($2X10^8$) 20 mg, *Bacillus mesentericus* TO-A ($2X10^6$) 20 mg, *Clostridium butyricum* TO -A ($2X10^6$) 20 mg and lactose 40 mg) to improve rumen function; Groviplex®, Ostovet®, Brotone® a cocktail of vitamin B complex, calcium, growth promoter at the rate of 5 ml/ day/ kid along with concentrate feed as a feed supplements.

Health care

Both the control and treatment group kids were given the same health cover like deworming and vaccination during the study period. During the study period, fecal samples were collected once in month and sent for screening of parasitic load. Common parasite detected in the fecal samples was *Schistosoma* sp. Based on the result the kids were dewormed using Ivermectin oral suspension at the rate of 0.02 mg/kg body weight once in a month.

Slaughter studies

At the end of the experiment, the animals were subjected to overnight fasting, recorded for their empty live weight and humanely slaughtered by the severance of carotid arteries and jugular veins. Slaughtering was carried out in a research abattoir at the Department of Meat Science, Madras Veterinary College and University Research Farm (TANUVAS).

After slaughter, the heads were removed at the atlanto-occipital joint, while the fore and hind legs were removed at the carpal and tarsal joints respectively. The animals were skinned while suspended by their achilles tendon. Carcass and non-carcass components were weighed immediately after slaughter. The heart, liver, spleen, kidney and lungs were weighed together and designated as pluck. The non-carcass components such as head, skin and feet were also weighed and designated as offal. The weight of digestive contents (gut fills) was computed as the difference between full and empty digestive tract (rumen and intestines). Prior to skinning and the removal of the visceral organs from the carcass, the oesophagus was tied with nylon string to prevent contamination of carcass by the gut contents. Visceral fats were removed and weighed. The carcasses were weighed immediately after dressing which was designated as hot carcass weight. Each carcass was split longitudinally to left and right halves. Each half was further split into fore and hind quarters using a carcass splitting saw and finally expressed as percentages of each tissue per whole carcass weight.

The amount of non-carcass components such as offal (head, legs and skin) was determined as a percentage of slaughter weight. The gut fill was recorded as percentages of total weight of gut (rumen and intestine including their contents) and the viscera (rumen and intestines) were reported as percentages of total weight of gut (including their contents) while the pluck (heart, liver, kidney and lungs) were weighed and recorded as percentages of carcass weight. The compositions of visceral fat, subcutaneous fat, inter-muscular fat as well as fat in pluck (heart, liver, kidney, and lungs) were recorded as percentages of total trimmable fats.

Collection and analysis of data

All the kids were weighed at fortnight interval. The average intake of concentrate feed, green and dry roughages by the kids were recorded daily. The carcass parameters such as pre slaughter weight, carcass weight, dressed weight and weights of blood, head, feet, stomach with contents, lungs, heart, kidney, spleen, liver and skin were also studied. The accumulated data were analyzed by 't' test using Graphpad prism software.

RESULTS AND DISCUSSION

Feeding high quality protein rich diet with added supplements for enhanced growth, feed efficiency and carcass quality is called accelerated feeding. Accelerated milk feeding system have been commonly used in calf rearing by supplementing high quantity of milk than conventional feeding for increased growth rate and earliest first calving. However, in goats accelerated feeding has been tried with either increasing the energy level of concentrate feed or protein level of concentrate feed. Saeed Ahmed Abbasi et al. (2012) have studied the effect of different dietary energy levels on the growth performance of Kamori goat kids. He concluded that high energy ration is cost effective and positively affects on weight gain and dressing percentage age of goat kids. So it can be used for increasing meat production. Liméa *et al.* (2009) have studied the growth performance and carcass quality of indigenous Caribbean goats under varying nutritional densities i.e. different protein content of the concentrate diet. Thus increasing the energy or protein level in the diet of goat kids consequently increases the growth and carcass quality. The current study was formulated to test the hypothesis that increasing the crude protein level in the diet of country goats will improve the growth and carcass quality of kids.

Proximate analysis of feed and fodder

The proximate analysis of concentrate feed, green fodder and dry fodder used in the study were given in table 2 and 3.

Table 2 : Proximate analysis of concentrate feed

Proximate analysis (%)	Concentrate ration for control group of kids	Concentrate ration for treatment group of kids
Moisture	15.18	11.08
Crude protein	15.18	21.02
Crude fiber	15.31	15.14
Ether Extract	2.58	2.58
Total ash	8.57	8.50
AIA	0.87	0.88
NFE	51.79	54.94

Table 3
Proximate analysis of green and dry fodder

Proximate analysis (%)	Sorghum dry fodder	Black gram tops dry fodder	Ground nut tops dry fodder	COFS29 grass	CO4 grass
Moisture	36.52	10.87	13.44	59.76	75.88
Crude protein	4.26	5.71	9.74	6.81	7.73
Crude fiber	28.06	50.38	33.31	24.28	27.71
Ether Extract	2.38	0.60	1.44	2.66	2.25
Total ash	10.41	6.60	6.39	14.56	13.99
AIA	-	-	1.56	-	-
NFE	54.89	36.71	49.12	50.19	48.32

Average feed intake

The average daily feed intake per goat during the study period is given in table 4. From the table it is evident that the kids of both the groups have taken almost similar quantity of feed and fodder throughout the study period.

Table 4
Average feed intake per goat in both control and treatment groups (n = 30)

Age	Concentrate (g) (Mean ± SE)	Green fodder (kg) (Mean ± SE)	Dry fodder (kg) (Mean ± SE)
60- 75 days	100	0.60± 0.05	0.20± 0.20
90 – 105 days	100	0.72± 0.04	0.25±0.02
120 – 135 days	100	0.76± 0.04	0.27± 0.03
150 – 165 days	100	0.84±0.04	0.28±0.10
180 – 195 days	100	1.20±0.08	0.42±0.18
210- 225 days	100	2.0±0.08	0.49±0.17

Body weight of kids

The fortnight body weight of kids under control and treatment group were given in table 5. From the table it is evident that the treatment and control group have almost equal weight at the start of the trial. However, the increased body weight in the treatment group is apparent during the course of the study.

Table 5
Body weight of kids

Age in days	Control (n=15)	Treatment (n=15)
	Body weight (kg) (Mean ± SE)	Body weight (kg) (Mean ± SE)
45 days	6.92±0.09	7.022±0.06
60 days	7.02±0.08	7.41±0.06
75 days	7.25±0.70	7.79±0.06
90 days	7.63±0.08	8.15±0.07
105 days	8.05±0.08	8.43±0.07
120 days	7.58±0.98	8.12±0.07
135 days	8.03±0.10	8.71±0.07
150 days	9.13±0.11	10.23±0.07
165 days	9.54±0.11	11.25±0.07
180 days	11.22±0.11	12.9±0.07
195 days	12.81±0.10	14.47±0.06
210 days	13.28±0.10	15.31±0.06
225 days	14.8±0.12	17.0±0.06

Production parameters

The growth parameters of the control and treatment group were given in table 6. Significantly higher total body weight gain (C - 6.74 ±0.09 kg,T - 9.98±0.10 kg) and daily body weight gain weight (C - 0.04±0.08 g, T - 0.06±0.09 g) was noticed in the treatment group fed with concentrate having 21% crude protein level than control group. Bhakt *et al.* (1987) also reported higher growth rate with increasing dietary crude protein level in the diet of goats and observed maximum growth rate fed with dietary crude protein level of 25% in indigenous Bihar goats. Significantly higher average daily weight gain was noticed in the treatment group kids i.e. 0.06±0.09 kg/day/kid than control group 0.04±0.08 kg/day/kid. Liméa *et al.* (2009) also reported that feeding diet containing 20.9% crude protein at 140g/kid/day, 240g/kid/day and 340g/kid/day significantly improve the average daily weight gain indigenous

Caribbean goats. The cost of feeding per kg live weight gain was significantly lower in the treatment group (Rs.72.48±0.12) than control group (Rs.98.15±0.15). Thus, accelerated feeding i.e. feeding high protein diet to kids improves its growth performance with significant reduction in the production cost.

Table 6
Production parameters and economics

Parameters	Control	Treatment
No. of kids per treatment	15	15
Initial body weight (kg) (30 - 45 days of age)	6.92±0.09	7.02±0.06^{NS}
Final body weight (kg) (210 - 225 days of age)	13.28±0.10	17.00±0.06**
Average total body weight gain (kg)	6.74 ±0.09	9.98±0.10**
Average daily body weight gain (kg/day/kid)	0.04±0.08	0.06±0.09*
Cost of production per kg live weight gain (Rs.)	98.15±0.15	72.48±0.12**

* - Significant (P<0.05), ** - Significant (P<0.01), ^{NS} - Not significant (P>0.05)

Slaughter studies

The carcass parameters studied were given in table 7. From the table, it is evident that the carcass quality of the accelerated feeding group kid was significantly higher than the control group kids. The high protein diet fed kids had significantly higher pre slaughter weight 17.0±0.06 kg, carcass weight 8.00±0.02 kg, dressed weight 7.55±0.14 kg and dressing percentage 47.06±0.12% than control group kids fed with convention feed with 15% dietary crude protein level. Limea *et al.* (2014) also found that the carcass weight and the dressing percentage of Creole kids improved with the progressive addition of concentrate with 20.9% crude protein at the rate of at G100 - 140g/kid/day, G200 - 240g/kid/day and G300 - 340g/kid/day. He also stated that the greater dressing percentage for G200 and G300 animals was probably due to better body development. Thus, accelerated feeding with high dietary crude protein level improves the carcass quality and body development in goats.

Table 7
Slaughter studies
Age of the carcass: 225 days

Parameters	Control (n=15) (Mean ± SE)	Treatment (n=15) (Mean ± SE)
Pre slaughter weight (kg)	14.8±0.12	17.0±0.06**
Carcass weight (kg)	6.25± 0.10	8.00±0.02**
Dressed Weight (kg)	5.70±0.15	7.55±0.14**
Dressing percentage (%)	42.22±0.13	47.06±0.12**
Blood (kg)	0.41±0.22	0.46±0.31 NS
Head (kg)	1.13±0.22	1.25±0.02**
Feet (kg)	0.51±0.29	0.52±0.27 NS
Stomach (kg)	5.10±0.26	6.35±0.2**
Lungs (kg)	0.25±0.02	0.28±0.21 NS
Heart (kg)	0.07±0.003	0.07±0.00 NS
Kidney (kg)	0.11±0.10	0.09±0.01 NS
Spleen (kg)	0.03±0.00	0.03±0.00 NS
Liver (kg)	0.27±0.25	0.28±0.00 NS
Skin (kg)	1.18±0.04	1.28±0.57 NS

* - Significant (P<0.05)
** - Significant (P<0.01)
NS - Not significant (P>0.05)

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Effect of exogenous enzymes supplementation on growth performance and histo morphology of duodenum of broilers fed cashew apple waste based diets*

P.Venkatramana[1], S. Senthil Murugan[2] and H.S. Patki[3],
Department of Animal Nutrition,
College of Veterinary and Animal Sciences, Pookode, Wayanad, Kerala, India.

ABSTRACT

The current experiment was conducted to study the histology of duodenum in broilers fed with different levels of inclusion of cashew apple waste (CAW) as well as enzymes. The study was carried out for a period of 42 days. A sum total of two hundred and ten day-old vencobb-400 broiler chicks were randomly divided into seven groups with three replicates of 10 chicks in each group. Group 1 (G1) received control diet prepared as per BIS (2007) recommendations. G2 and G3 birds received diet prepared with 5 and 10 per cent CAW without any enzyme supplementation, respectively. G4 and G5 birds received 5 per cent CAW with supplementation of 500 g/ton and 750 g/ton of enzymes, respectively. G6 and G7 birds received 10 per cent CAW with supplementation of 500 g/ton and 750 g/ton of enzymes, respectively. It was found that, histomorphological features like villus height and thickness of tunica mucosa were found to be higher in group (G4) where 5 per cent CAW with NSP degrading enzymes at 500 g/ton were fed significantly ($p<0.01$) when compared to all other groups. The number of goblet cells was observed to be significantly ($p<0.01$) lesser in the group (G4) when compared to all other groups.

Keywords: Cashew apple waste, duodenum, enzymes, goblet cell, villus

INTRODUCTION

Cashew apple waste (CAW) a by-product of cashew apple processing has been identified as an alternative feed resource for poultry but wasted without commercial exploitation (Murugan *et al.*, 2015). Though, CAW is considered as one of the feed resources and shows promising growth performance in broilers (Bhamare *et al.*, 2016); inclusion levels are limited due to presence of anti nutritional factors. Due consideration were given to identify anti nutritional factors such as level of condensed tannins and non starch polysaccharides present in CAW (Murugan *et al.*, 2015; Bhamare *et al.*, 2016 and Venkatramana *et al*., 2018) and these anti nutritional factors were reported to reduce digestibility and influence the growth performance of poultry (Choct, 2006). There are studies on dietary non starch polysaccharides which increases small intestine fermentation and affects the nutrient digestion and absorption for chickens (Bharathidhasan *et al*., 2010; Nian

Corresponding author email id: *Part of M.V.Sc thesis of the first author submitted to Kerala Veterinary and Animal Sciences University, Pookode, Kerala.
1. MVSc Student 2. Assistant Professor
3. Assistant Professor, Dept. of Anatomy

et al., 2011). However currently exogenous enzymes are used in poultry diets to improve the quality of feed ingredients used. The mechanism by which exogenous enzymes improves the growth performance of broilers could be through direct effect on endogenous enzyme activity (Yuan *et al.*, 2008); partial hydrolysis of the non starch polysaccharides (Zhang *et al*., 2014); enhancing nutrient absorption by increasing the villus height in the small intestine (Panda *et al.,* 2006); modifying mucin biosynthesis and or degradation, which in turn influences gut function resulting in improved nutrient uptake (Smirnov *et al*.,2005). Duodenum is the first segment of the small intestine where the process of digestion and trace amount of absorption occurs. Thus, the changes in the duodenum histomorphology might provide idea about actions of exogenous enzymes in cashew apple waste based diets. Thus this study was undertaken to study the histology of duodenum in broilers fed on dietary supplementation of cashew apple waste and at different inclusion levels of enzymes.

MATERIALS AND METHODS

Birds, diets and experimental design

A total of 210 one-day old commercial broiler chicks (Vencobb-400) were purchased from local hatchery. The chicks were weighed individually, wing banded and randomly distributed to seven groups *viz.,* G1, G2, G3, G4, G5, G6 and G7 with three replicates of ten chicks in each group. The study was conducted in Poultry Farm, Instructional Livestock Farm complex (ILFC), Pookode and facilities available in Department of Animal Nutrition and Department of Veterinary Anatomy and Histology at College of Veterinary and Animal Sciences, Pookode, Wayanad were utilized. The broiler chicks were fed with broiler pre-starter for first seven days then broiler starter feed was fed from 8th day to 20th day and followed by broiler finisher feed. The control diet was corn soya bean based without enzyme. The cocktail exogenous degrading enzymes with composition of amylase (24,00,000 Units/kg), hemicellulase (54,00,000 Units/kg), cellulase (1,20,00,000 Units/kg), beta-glucanase (1,06,000 Units/kg) and protease (24,00,000 Units/kg) were used in this study. All the experimental diets were formulated with CAW to meet nutrient requirements mentioned in BIS (IS: 1374; 2007). The experimental design of this study is presented in table-1. Growth Performance of the experimental birds was measured by recording body weight at weekly interval.

Table1. Experimental design

Group	Inclusion level of CAW (%)	Supplementation level of cocktail enzymes (g/ton)
G1	0	0
G2	5	0
G3	10	0
G4	5	500
G5	5	750
G6	10	500
G7	10	750

Histo-morphological study

At 42nd day of the trial six birds from each group were sacrificed after administrating chloroform anaesthesia and representative pieces from duodenum were collected and fixed using 10 per cent neutral buffered formalin for histo-morphological measurements. The paraffin embedding was done in medium paraffin wax with ceresin and tissue sections were taken at 5 µm thickness by using semi-automatic M-TECH microtome. Haematoxylin and Eosin (H & E) and Per Iodic Acid Schiff (PAS) staining methods were used to study histological and muco-polysaccharides respectively as per Luna (1968). The micrometric measurements were taken using ProgRes® capture 2.8.8.version. JENOPTIKR, Optronics software at 10 X magnification for intestinal villus, thickness of mucosa and 40 X magnification for goblet cells and height of epithelium.

Statistical analysis

The data were analyzed using GLM procedure of statistical Package for social sciences (SPSS) 21st version and comparison of means was done using Ducan's multiple range test and significance was considered at $p < 0.01$.

RESULTS AND DISCUSSION

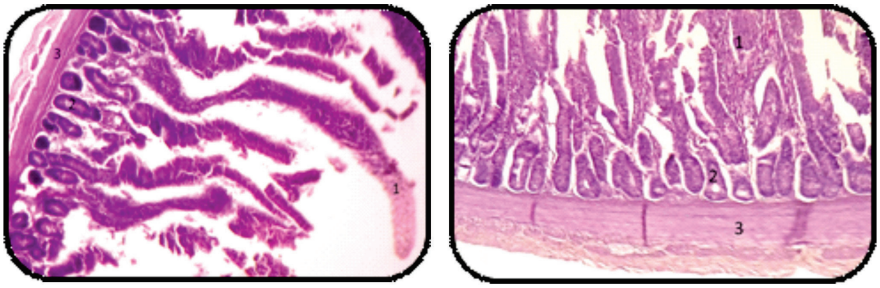
Inclusion of CAW at 5 per cent without NSP degrading enzyme did not show any improved body weight in this study; whereas Bhamare *et al*. (2016) mentioned better body weight compared against 10 and 20 per cent inclusion level in broilers. Significant increase in live body weight (g/bird) of broilers in CAW (5 per cent) with enzyme supplementation (500 g/ton) at 6th week of its age where recorded when compared against 5 and 10 per cent CAW fed without enzyme supplementation. In this study, the enzyme supplemented groups showed improved body weight comparable to control. The mean weekly body weight of the broilers is presented in table-2.

Table 2. Mean weekly body weight of broilers (g/bird) fed CAW with/without enzyme supplementation

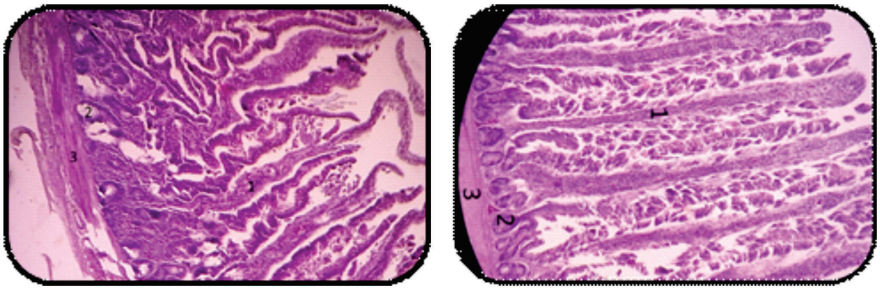
Age (week)	G1	G2	G3	G4	G5	G6	G7	F-value	p-value
Initial B. wt	42.36 ± 0.47	44.72 ± 2.10	44.24 ± 0.68	44.03 ± 0.46	45.48 ± 1.03	43.99 ± 0.64	46.14 ± 0.68	1.42 ns	0.27
I	150.58^{b} ± 0.59	143.39^{c} ± 0.66	161.24^{a} ± 1.71	154.01^{b} ± 0.30	155.34^{b} ± 3.37	165.33^{a} ± 1.63	150.89^{b} ± 2.11	16.52**	<0.001
II	391.82^{a} ± 6.33	319.56^{b} ± 36.44	407.14^{a} ± 15.45	440.00^{a} ± 1.69	417.20^{a} ± 1.11	421.66^{a} ± 6.55	417.31^{a} ± 7.46	6.29**	<0.001
III	742.52^{ab} ± 6.91	671.02^{c} ± 8.28	731.73^{ab} ± 24.58	698.02^{bc} ± 3.67	715.99^{abc} ± 8.97	759.50^{a} ± 27.45	764.40^{a} ± 2.85	5.03**	<0.001
IV	1312.58^{ab} ± 54.78	1268.14^{b} ± 10.78	1322.00^{ab} ± 41.01	1414.37^{a} ± 35.17	1262.52^{b} ± 35.91	1437.75^{a} ± 59.45	1369.88^{ab} ± 26.48	2.84*	<0.05
V	1894.26^{b} ± 4.68	1833.26^{b} ± 10.63	1877.33^{b} ± 38.69	2061.90^{a} ± 4.47	1937.65^{b} ± 27.32	1910.89^{b} ± 47.62	1929.72^{b} ± 56.59	4.54**	<0.001
VI	2290.87^{abc} ± 35.68	2194.99^{bc} ± 56.79	2264.33^{bc} ± 35.57	2395.08^{a} ± 5.99	2365.93^{a} ± 9.61	2268.33^{bc} ± 53.22	2288.89^{abc} ± 20.96	3.40*	<0.02

Mean values with different superscript within a row differ significantly.** Significant at 0.01 level; * significant at 0.05 level; ns-non- significant. G1-without CAW and cocktail enzymes; G2- 5 % CAW without cocktail enzymes; G3- 10% CAW without cocktail enzymes; G4-5 % CAW with cocktail enzymes (500 g/ton); G5-5 % CAW with cocktail enzymes (750 g/ton); G6- 10 % CAW with cocktail enzymes (500 g/ton);G7- 0 % CAW with cocktail enzymes (750 g/ton).

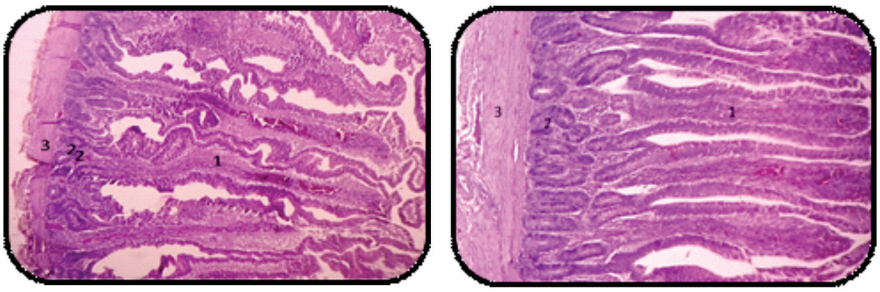
The micrographs depicting histo architecture of villi are as shown in Fig. 1. Per Iodic Acid Schiff stain micrographs of goblets cells are depicted in Fig.2. The micrometric measurements of duodenum length of villus (µm), thickness of tunica mucosa (µm) and goblet cell numbers recorded are presented in table 3.



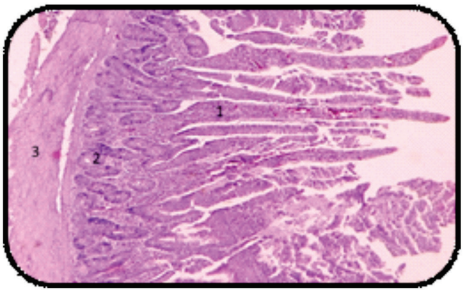
G I G II



G III G IV



G V G VI



G VII

Figure 1: Microphotographs of Duodenum in G I to G VII (Haematoxylin and Eosin method X 100)

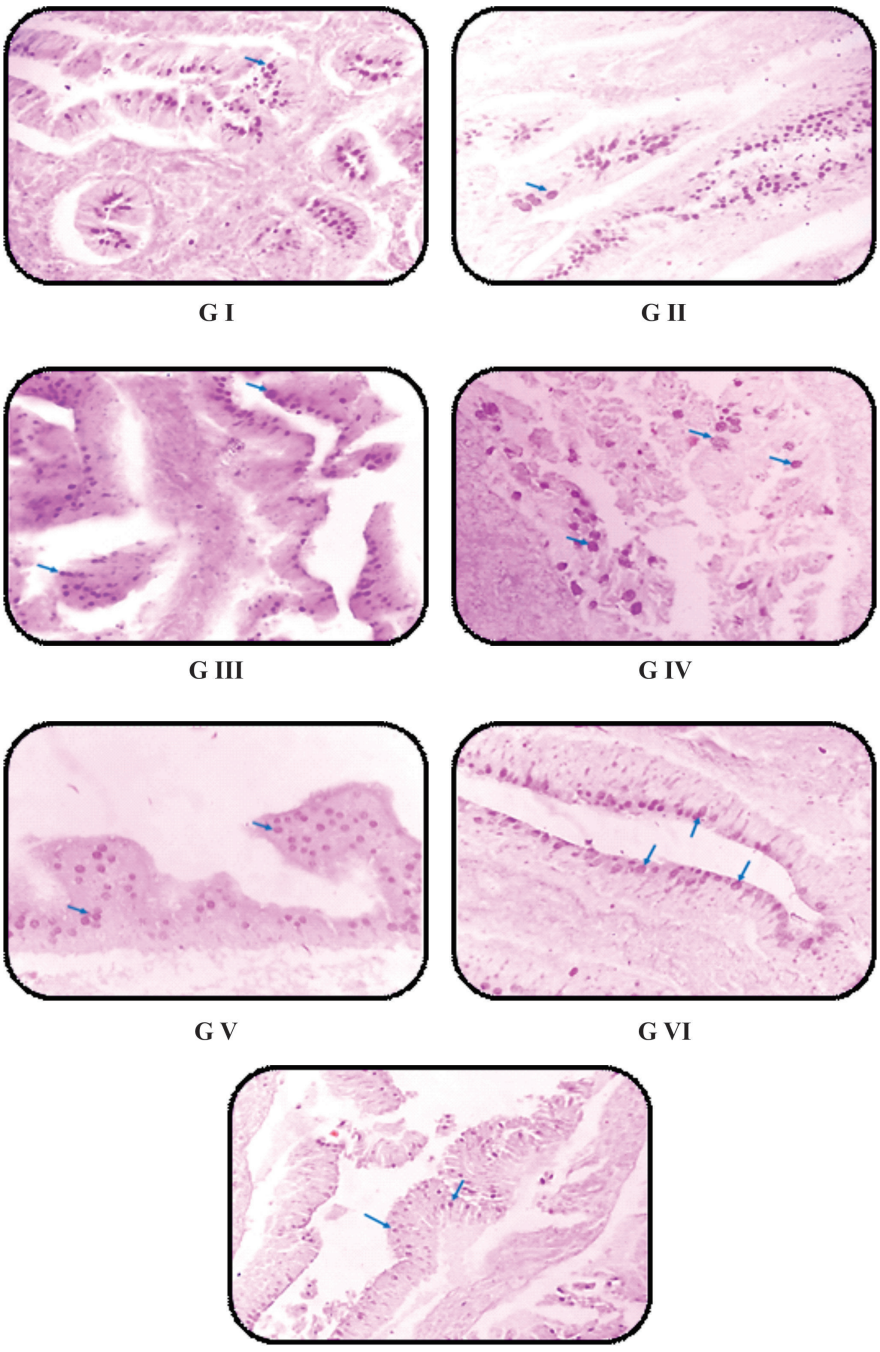


Figure 2: Microphotographs of Duodenum in G I to G VII (Per iodic Acid Schiff method X 400) Arrow indicates goblet cells showing presence of mucopolysaccharides

Table 3.Micrometric parameters of duodenum

Attributes	Group							F-value	p-value
	G1	G2	G3	G4	G5	G6	G7		
Villi height (µm)(10X)	1292.43^{bc} ± 47.55	1389.82^{b} ± 32.45	934.63^{e} ± 24.96	1601.08^{a} ± 71.13	1180.33^{cd} ± 28.79	1262.43^{bcd} ± 34.26	1154.47^{d} ± 50.26	22.22**	<0.001
Thickness of mucosa (µm) (10X)	1522.10^{abc} ± 68.68	1688.08^{ab} ± 18.93	983.60^{d} ± 161.30	1779.43^{a} ± 37.72	1420.6^{bc} ± 31.88	1293.1^{cd} ± 225.84	1413.70^{bc} ± 38.97	5.61**	<0.001
Goblet cell number (40X)	267.00^{a} ± 3.10	203.83^{b} ± 3.82	193.83^{c} ± 3.20	89.17f ± 2.89	107.83^{e} ± 2.41	113.00^{e} ± 2.67	167.67^{d} ± 2.80	448.35**	<0.001
Height of Lining epithelium (µm) (40X)	38.60^{a} ± 0.80	24.83^{d} ± 0.71	31.50^{c} ± 0.67	30.85^{c} ± 0.65	35.46^{b} ± 1.45	33.20^{bc} ± 1.27	35.03^{b} ± 1.43	16.99**	<0.001

Mean values with different superscripts with in a row differ significantly.** Significant at 0.01 level.
G1-without CAW and cocktail enzymes;G2- 5 % CAW without cocktail enzymes;G3- 10% CAW without cocktail enzymes; G4-5 % CAW with cocktail enzymes (500 g/ton);G5-5 % CAW with cocktail enzymes (750 g/ton);G6- 10 % CAW with cocktail enzymes (500 g/ton);G7- 0 % CAW with cocktail enzymes (750 g/ton).

Significant ($p<0.01$) increase in villi length of duodenum in enzyme supplemented group in current study are in concurrence with Balamurugan *et al*.(2011); Mazhari *et al*.(2015) and Thavasiappan *et al*. (2016).The highest duodenal villus length was recorded in group (G4) where 5 per cent CAW with NSP degrading enzymes at 500 g/ton was fed ($p<0.01$). However, there was no linear increase in villi length, but a decrease was recorded in 750 g/ton supplemented group with 5 per cent CAW. Similar, observations were given by Yuan *et al.* (2008) and Luo *et al*. (2009) where higher dose of enzyme might suppress excretion of endogenous enzymes and damage the structure of small intestine.

Significantly ($p<0.01$) minimum number of goblet cells have been recorded in duodenum of birds that received 5 per cent CAW with 500 g/ton of enzyme supplementation. Similarly, Balamurugan *et al*. (2011) recorded reduced goblet cell numbers compared to control in broiler chicks where cellulase, xylanase, pectinase and phytase enzyme were supplemented.

It could be corroborated that, the birds fed with 5 per cent CAW, supplemented with 500 g/ton enzymes showed increase in duodenum villi length which, increased surface area and allows greater absorption of nutrients. Decrease in goblet cells numbers in response to enzyme supplementation evidenced disruption of mucous layers, decrease in mucin secretion, improved digestibility and better body weight.

CONCLUSION

It could be concluded that the cashew apple waste included in broiler diet and enzyme supplementation improves bird's performance which correlated with morphometric measurements of duodenum.

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Development of liquid milk replacer for rearing early weaned piglets

K. Roopa, R. Karunakaran, D.Balasubramanyam, H. Gopi and L.Radhakrishnan
Tamil Nadu Veterinary and Animal Sciences University.
Department of Animal Nutrition
Madras Veterinary College, Chennai - 600 007, India

ABSTRACT

A 28 day feeding trial was conducted to assess the effect of liquid milk replacer on the growth performance of early weaned piglets. Forty piglets (average birth weight 1.18 ± 0.01 kg) of 14 days of age were selected and grouped into five treatments groups viz (control, unweaned), T_1, T_2, T_3 T_4 and T_5 and fed with liquid milk replacer type I, type II, type III and type IV, respectively. This study revealed that piglets supplemented with liquid milk replacer type I (T_2) had comparable body weight gain (5.95 ± 0.08 kg) compared to control (5.77 ± 0.28 kg) and T_3, T_4 and T_5 had significantly lower weight gain than T_2 and control piglets.

Key words: Average daily gain, Feeding trial, Milk replacer, Piglets,

INTRODUCTION

A newborn piglet lacks a fully developed immune system since they are born immature and they have never been exposed to antigens (Rooke and Bland, 2002). The rapid development of the neonate coincides with the rapid changes in composition of mammary secretions consumed by the suckling piglet. Sow milk production is the major factor limiting piglet growth prior to weaning. Weaning weight is quite variable from litter to litter and much of this variation is due to the quantity of milk produced by sows. Due to larger litter sizes and increased competition for sow milk, nutrient availability for newly born piglets is often limited (Kyriazakis *et al.,* 2006).

Milk replacer may also be offered to piglets while they are with the sow in the farrowing crate to increase weaning weights and reduce variation in weaning weight and mortality in early weaning programme (Hurley, 2016). Hence, an attempt was made in the present study to develop liquid milk replacer for rearing of early weaned piglets.

MATERIALS AND METHODS

The study was undertaken at the Pig Breeding Unit of Post Graduate Research Institute in Animal Sciences (PGRIAS), Kattupakkam, Kanchipuram district, Tamil Nadu. The research station is located in the longitude of 80.0395° E and latitude of 12.8259°N.

Liquid milk replacer preparation

Four types of liquid milk replacers were prepared by fortifying the cow milk with the deficit nutrients**.** Based on the analyzed chemical composition of sow milk and the cow milk as per AOAC (2016) the

deficit nutrients in cow milk is calculated as follows

% Difference in nutrients = % of Nutrients in Sow milk - % of Nutrients in Cow milk.

The deficit nutrients were supplied by using skim milk powder, whey protein, ghee and coconut oil (Table 1). The prepared liquid milk replacer was boiled and cooled at 35-37 ^{o}C then fed to piglets eight times in day viz 7 am, 9 am, 11 am, 1 pm, 3 pm, 5 pm, 6 pm and 8 pm. The liquid milk replacer intake was determined as per Thodberg *et.al*. (2006) and Skok *et. al*. (2007). Creep feed was offered *ad libitum* from 21 days of age to all the treatment groups.

Table 1 Ingredients Composition of liquid milk replacers (g/100g)

Types of milk replacers	Ingredients Composition of milk replacers				
	Cow milk, ml	Skim milk powder, g	Whey protein, g	Coconut oil, g	Ghee, g
Type I	100	4.8	-	-	1.75
Type II	100	4.8	-	1.75	-
Type III	100	-	2.1	-	1.75
Type IV	100	-	2.1	1.75	-

Animal feeding trial

The prepared liquid milk replacers were tested with the piglets from five crossbred sows (Tamil Nadu veterinary and Animal Science University Kattupakkam Gold) with a litter size of eight. Five treatments were formed in which treatment one (T_1) was kept control where the piglets were not separated from sow. The piglets from each of the other four sows were weaned by 14 days of age and assigned to one of the treatment two (T_2), three (T_3), four (T_4) and five (T_5) and fed with liquid milk replacer type I, type II, type III and type IV respectively. The weekly body weights were recorded and weekly body weight gain was calculated.

Statistical analysis

The data on weekly body weight and body weight gain were statistically analyzed using Analysis of Variance as per Snedecor and Cochran (1994). Means were compared by Duncan multiple range test using SPSS package of version 20 for windows.

RESULTS AND DISCUSSION

The data on proximate composition of cow milk and sow milk used for calculating the deficit nutrients were as follows. The present chemical composition of sow milk and cow milk for total solid, total ash, crude protein, fat, lactose and SNF was 20.81 ± 0.41 and 14.48 ± 0.37, 0.82 ± 0.04 and 0.71 ± 0.02, 5.01 ± 0.16 and 3.33 ± 0.12, 8.25 ± 0.34 and 4.74 ± 0.03, 6.25 ± 0.27 and 5.69 ± 0.29 and 12.64 ± 0.21 and 9.7 ± 0.35, respectively. The proximate composition results of cow milk and sow milk were in agreement with the findings of Scnurn (1968) and Hamad (2010).

The overall body weight of piglets at the start of the feeding trial in T_1, T_2, T_3, T_4 and T_5 were 3.68 ± 0.11, 3.40 ± 0.04, 2.89 ± 0.12, 4.08 ± 0.27and 4.07 ± 0.37 respectively. The average body weight of piglets at the end of the feeding trial in T_1, T_2, T_3, T_4 and T_5 were 9.45 ± 0.33, 9.36 ± 0.07, 8 ± 0.60, 8.29 ± 0.60 and 8.39 ± 0.52 respectively (Table 2). The body weight gain at the end of trial in T_1, T_2, T_3, T_4 and T_5 were 206.18 ± 9.83, 212.59 ± 2.71, 182.54 ± 9.9 150.36 ± 17.73 and 154.24 ± 9.12 respectively (Table 3).

Table 2 The effect of liquid milk replacer on the weekly body weight (kg) of piglets.

Days	T_1	T_2 (cow milk+ skim milk powder + ghee)	T_3 (cow milk+ skim milk powder + coconut oil)	T_4 (cow milk+ whey protein + ghee)	T_5 (cow milk+ whey protein + coconut oil)
Birth weight	1.20 ± 0.02	1.13 ± 0.01	1.16 ± 0.02	1.22 ± 0.04	1.23 ± 0.04
14	3.68 ± 0.11^{bc}	3.40 ± 0.04^{ab}	2.89 ± 0.12^{a}	4.08 ± 0.27^{c}	4.07 ± 0.37^{c}
21	4.90 ± 0.15^{ab}	4.57 ± 0.08^{ab}	4.16 ± 0.24^{a}	5.18 V 0.38^{b}	4.90 ± 0.33^{ab}
28^{NS}	6.39 ± 0.22	6.26 ± 0.11	5.64 ± 0.23	5.79 ± 0.38	5.69 ± 0.39
35	8.05 ± 0.24^{b}	7.88 ± 0.04^{b}	6.58 ± 0.18^{a}	6.83 ± 0.39^{a}	6.86 ± 0.40^{a}
42	9.45 ± 0.33^{b}	9.36 ± 0.07^{b}	8 ± 0.25^{a}	8.29 ± 0.60^{ab}	8.39 ± 0.52^{ab}

P≤0.05, Means with different superscripts in a row differ significantly

Table 3 The effect of liquid milk replacer on the cumulative body weight gain of piglets.

Weeks	T_1	T_2	T_3	T_4	T_5
1	1.22 ± 0.06^{ab}	1.17 ± 0.08^{ab}	1.27 ± 0.19^{b}	1.10 ± 0.15^{ab}	0.83 ± 0.13^{a}
2	2.71 ± 0.13^{b}	2.85 ± 0.12^{b}	2.75 ± 0.24^{b}	1.71 ± 0.21^{a}	1.62 ± 0.20^{a}
3	4.37 ± 0.17^{b}	4.47 ± 0.03^{b}	3.69 ± 0.22^{b}	2.75 ± 0.24^{a}	2.79 ± 0.22^{a}
4	5.77 ± 0.28^{b}	5.95 ± 0.08^{b}	5.11 ± 0.28^{ab}	4.21 ± 0.50^{a}	4.32 ± 0.26^{a}

P≤0.05, Means with different superscripts in a row differ significantly

The piglets fed with liquid milk replacer type I have gained 5.95 ± 0.08 g of body weight when compared with other treatments. There was no significant (P<0.05) difference in body weight and body weight gain between the treatments. This was in agreement with the findings of Ruurd *et.al*. (1996). However, Dunshea *et.al*. (1999) found that skim milk feeding before and after weaning could result in cumulative improvements in growth performance in the nursing piglets. André *et. al.* (2005) also observed that feeding whey protein as source of protein increased the weight gain by 20 percent when compared with the vegetable protein source. Richard *et. al.*(1990) concluded that there was no difference in the average daily weight gain between the semiautomatic feeding system group and conventionally fed group, but the diarrhea was commonly seen in conventional system of rearing. Azain *et.al.* (1996) fed commercial milk replacer on fresh basis (150g/L) *ad libitum* till

weaning (d 21) and observed that the average pig weight and total litter weight at weaning there was no significant difference in litters receiving supplemental milk replacer.

Based on the results obtained from the present study it is observed that there was a significant reduction in the weight gain in the treatments T_3, T_4 and $T_5$when compared to the T_1(control) and T_2. However there was no significant difference between the T_1 (control) and T_2 in body weight and body weight gain at 42nd day of weaning age. Piglets supplemented with liquid milk replacer type I has shown comparable body weight and body weight gain with that of piglets reared with sow's milk (control).

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Development and quality evaluation of low fat functional paneer

Kavya Kuttan and K. Radha
Kerala Veterinary and Animal Sciences University
Department of Dairy Science,
College of Veterinary and Animal sciences, Mannuthy-680651, Kerala, India

ABSTRACT

An experiment was conducted to utilize skim milk for the preparation of low fat paneer, a value added product. Low fat paneer was prepared by using whey protein concentrate (WPC) and inulin as fat replacers. Full fat control paneer (C1) was prepared from buffalo milk standardized to 5 per cent fat and 9 per cent SNF. Skim milk control paneer (C2) was prepared from skim milk standardized to 0.3 per cent fat and 9 per cent SNF. WPC was incorporated at 0.25 (T1) and 0.5 per cent levels (T2). Inulin was used at the level of 1.5 (T1) and 2 per cent (T2) for the preparation of low fat paneer. The prepared paneer samples were evaluated for physico-chemical, microbiological and organoleptic qualities. Incorporation of WPC had significantly increased the yield as well as moisture content of low fat paneer. Inulin at 2 per cent level had increased the yield of low fat paneer. Both WPC and inulin had improved the sensory acceptability of low fat paneer prepared from skim milk.

Key words: Paneer, Whey protein concentrate, Inulin, Low fat paneer, Fat replacers

INTRODUCTION

Milk has long been recognized as an ideal and nearly perfect food for all sections of the society. Paneer is an indigenous dairy product obtained by acid coagulation of hot whole milk with subsequent drainage of whey. It is a rich source of milk protein and fat available at a comparatively lower cost. However, it is not preferred by the modern health conscious consumers due to its high fat content. As per the data published by WHO (1965), high intake of fat rich dairy products increased the risk of cardio vascular diseases because of high proportions of saturated fatty acids. This offers an opportunity for development and commercial manufacture of low fat paneer suitable for persons suffering from lifestyle related diseases. Since fat is largely responsible for the desirable flavor, body and texture of paneer, reducing the fat content will lead to several sensory quality defects such as rubbery, chewy and hard body. These defects could be prevented by the use of fat replacers in low fat products.

Fat replacers are the substances with the same functions, stability, physical and chemical characteristics as regular fat but with low calories. They help to improve the organoleptic qualities of low fat dairy products. Whey protein concentrates (WPC) are protein based fat replacers that have the potential to improve the organoleptic qualities of low fat dairy products. Inulin is a carbohydrate based fat replacer and dietary fiber extracted from chicory root. It also improves the flavor, body and texture of low-fat foods.

MATERIALS AND METHODS

Preparation of paneer

Paneer was prepared as per the procedure suggested by Sachdeva (1983). Milk was heated to 90°C and promptly cooled to 70°C. Then it was coagulated by using 1 per cent citric acid. Then the coagulum was pressed and the yield of paneer was recorded. Full fat control paneer was prepared from milk standardized to 5 per cent fat and 9 per cent SNF (C1). Skim milk control paneer (C2) was prepared from skim milk standardized to 0.3 per cent fat and 9 per cent SNF. Low fat paneer was prepared by incorporating WPC at 0.25 (T1) and 0.5 per cent (T2) levels. Inulin was incorporated at 1.5 (T1) and 2 per cent (T2) levels for the preparation of low fat paneer.

Analysis of the paneer

Chemical analysis

Control and treatment groups of paneer were analyzed for yield, fat, moisture, total solids, and titratable acidity. The fat percent of paneer was analyzed by the procedure suggested by BIS (1977). Moisture and total solids contents of paneer were determined as per the procedure described in BIS (1983). The titratable acidity was determined as per AOAC (2000).

Microbiological quality of paneer

Standard plate count, coliform count and yeast and mould count of paneer samples were determined according to the procedure described by BIS (1980).

Sensory evaluation

The fresh paneer samples were evaluated for their sensory characteristics such as color and appearance, flavor, body and texture and overall acceptability as per the method recommended by BIS (2003). The data obtained from various studies were subjected to statistical analysis by following the procedure described by Snedecor and Cochran (1994). Six replications were done in each category. The statistical studies were carried out for comparing physical, chemical, microbiological and sensory parameters of low fat paneer with control. Duncan's Multiple Range Test (DMRT) was carried out for pair wise comparison if 'F' values are found to be significant in ANOVA.

RESULTS AND DISCUSSION

Physico-chemical quality of low fat paneer incorporated with whey protein concentrate

Table 1 represents the physico-chemical analysis of low fat paneer incorporated with whey protein concentrate

Table 1: Physico- chemical analysis of low fat paneer incorporated with WPC (Mean±S.E)

Parameters (%)	Full fat control(C1)	Skim milk control(C2)	Skim milk paneer incorporated with 0.25%WPC(T1)	Skim milk paneer incorporated with 0.5%WPC(T2)
Yield	18.00±0.50^{a}	13.46±0.57^{b}	14.21±0.61^{bc}	15.73±0.61^{c}
Fat	19.42±0.33^{a}	4.58±0.20^{b}	4.17±0.33^{b}	4.25±0.21^{b}
Moisture	54.53±0.37^{a}	56.56±0.74^{a}	55.43±0.51^{a}	59.03±1.25^{b}
Total solids	45.47±0.37^{a}	43.43±0.73^{a}	44.53±0.50^{a}	39.78±1.19^{b}
Titratable acidity	0.43±0.03^{a}	0.47±0.02^{a}	0.45±0.02^{a}	0.47±0.02^{a}

Means within a row bearing different letters as superscript differ significantly.

Means within a row bearing same letters as superscript are homogenous.

Yield

The mean yield of control and treatment groups of (C1, C2, T1 and T2) paneer was 18.00±0.50, 13.46±0.57, 14.21±0.61, 15.73±0.61 respectively. The yield of treatment groups of paneer and skim milk control paneer were significantly lower than full fat control paneer. Addition of WPC had increased the yield of paneer. Lo and Bastion (1998) had reported similar results in low-fat Haverti cheese.

Fat

The mean fat percentage of control and treatment groups of paneer samples were 19.42±0.33 (C1), 4.58±0.20 (C2), 4.17±0.33 (T1) and 4.25±0.21 (T2) respectively. The fat content of treatment groups of paneer and skim milk control was significantly lower than full fat control. Incorporation of WPC had reduced the fat content of treatment groups of paneer when compared to skim milk paneer. Bhatt (2013), observed that there was a decrease in fat recovery with increase in the level of WPC in low fat paneer.

Moisture

The mean moisture content of paneer samples were 54±0.37 (C1), 56.56±0.74 (C2), 55.43±0.51 (T1) and 59.03±1.25 (T2) per cent respectively. There was a significant difference in moisture content between treatment groups and full fat control paneer. Skim milk control paneer (C2) and T1 did not show any significant difference in moisture content whereas, T2 showed significant variation from C2 and T1. Narayanarao (2005) had reported an increase in moisture content in low fat paneer incorporated with whey protein concentrate. In this study also incorporation of WPC at 0.5 per cent level had significantly increased the moisture content of paneer.

Total solids

The mean values of total solids per cent in paneer samples were 45.47±0.37 (C1), 43.43±0.73 (C2), 44.53±0.50 (T1) and 39.78±1.19 (T2) respectively. The total solids content in treatment groups and skim milk control paneer were significantly different from full fat control. Bhatt (2013) had reported that there was an increase in

total solid recovery in low fat paneer when WPC was incorporated at the rate of 0.2 per cent. However, higher levels of addition of WPC resulted in decrease in total solid recovery.

In the present study also addition of WPC at 0.25 per cent had slightly increased the total solid content but incorporation at 0.5 per cent level had decreased the total solids content.

Titratable acidity

The mean titratable acidity of full fat and skim milk control paneer were 0.43±0.03 and 0.47±0.02 per cent lactic acid respectively. The titratable acidity of treatment groups of paneer were 0.45±0.02 and 0.47±0.02 per cent lactic acid respectively. There was no significant difference in titratable acidity between control and treatment groups of paneer. Narayanarao (2005) reported that the pH and titratable acidity in low fat paneer did not differ much by the addition of WPC.

Microbiological quality

Table 2 represents the results of microbiological analysis of low fat paneer incorporated with whey protein concentrate

Table 2: Microbiological quality of low fat paneer incorporated with WPC (Mean±S.E)

Microbiological analysis	Storage days	Full fat control (C1)	Skim milk control (C2)	Skim milk paneer incorporate with 0.25%WPC (T1)	Skim milk paneer incorporated with 0.5%WPC (T2)
SPC	0th day	3.98±0.05^{a}	4.05±0.02^{a}	3.98±0.05^{a}	3.99±0.04^{a}
	4th day	4.14±0.03^{b}	4.11±0.04^{a}	4.08±0.02^{a}	4.09±0.04^{a}
	7th day	4.25±0.01^{c}	4.23±0.02^{b}	4.19±0.03^{b}	4.21±0.02^{b}
Yeast and mould	0th day	1.15±0.07^{a}	1.26±0.09^{a}	1.23±0.08^{a}	1.13±0.09^{a}
	4th day	1.48±0.06^{a}	1.38±0.09^{a}	1.39±1.39^{a}	1.35±0.09^{a}
	7th day	1.57±0.04^{a}	1.45±0.07^{a}	1.59±0.08^{a}	1.60±0.03^{a}
Coliform count	0th day	0.96±0.21^{a}	0.72±0.23^{b}	0.88±0.18^{a}	0.72±0.23^{b}
	4th day	0.22±0.22^{a}	0	0.33±0.33^{ab}	0.23±0.17^{a}
	7th day	0	0	0	0

Means within a row bearing different letters as superscript differ significantly.

Means within a row bearing same letters as superscript are homogenous.

Standard plate count

The mean standard plate count of full fat control paneer (C1) was 3.98±0.05, 4.14±0.03 and 4.25±0.01 log cfu/g respectively for the 0th, 4th and 7th day of storage. For C2 paneer the values were 4.05±0.02, 4.11±0.04 and 4.23±0.02 log cfu/g respectively. The values for T1 paneer were 3.98±0.05, 4.08±0.02 and 4.19±0.03 log cfu/g respectively. For T2 paneer, the mean values were 3.99±0.04, 4.09±0.04and 4.21±0.02 log cfu/g respectively. A progressive increase in SPC was noticed during storage in all paneer samples. However, the counts were within the legal limit until seven days of storage.

Yeast and Mould count

The mean yeast and mould count of full fat paneer was 1.15±0.07, 1.48±0.06 and 1.57±0.04 logcfu/g for the 0th, 4th and 7th day of storage respectively. For C2 the counts were1.26±0.09, 1.38±0.09 and 1.45±0.07 log cfu/g respectively. For treatment group one (T1) the mean values were 1.23±0.08, 1.39±1.39 and 1.59±0.08 log cfu/g respectively. In treatment group two (T2), the mean values were 1.13±0.09, 1.35±0.09 and 1.60±0.03 log cfu/g respectively. Addition of WPC had not altered the yeast and mould count of paneer. Even though the yeast and mould count increased during storage, it was within the legal limit until seven days of refrigerated storage in all groups of paneer samples.

Coliform count

The coliform count of paneer samples were taken during the 0th, 4th and 7thday of refrigerated storage. Coliform count were absent in all paneer samples on 7th day of storage. The coliform count on 0thand 4th day of storage in full fat control paneer (C1) were 0.96±0.21 and 0.22±0.22 log cfu/g respectively. For C2 paneer, the values were 0.72±0.23 and 0.00±0.00 log cfu/g respectively. For T1 paneer the values were 0.88±0.18 and 0.33±0.33 log cfu/g respectively. For T2 the values were 0.72±0.23 and 0.23±0.17 log cfu/g respectively. Coliform count did not show any significant difference between control and treatment groups of paneer. It was within the acceptable limit prescribed by FSSA until seven days of refrigerated storage. The absence of coliform organisms on 7th day of storage might be due to developed acidity which might have prevented the growth of coliform.

Sensory evaluation

Table 3 represents the sensory scores of low fat paneer incorporated with whey protein concentrate

Table3. Sensory scores of WPC added low fat paneer(Mean ± S.E)

Parameters	Full fat control(C1)	Skim milk control (C2)	Skim milk paneer incorporate with 0.25%WPC (T1)	Skim milk paneer incorporated with 0.5%WPC (T2)
Color and appearance	9.6±0.21^{a}	6.83±0.40^{b}	8.00±0.45^{c}	8.33±0.33^{c}
Body and texture	39.17±0.40^{a}	31.17±0.91^{b}	34.33±1.38^{bc}	35.00±1.63^{c}
Flavour	48.33±0.71^{a}	42.00±1.51^{a}	45.17±1.11^{a}	45.83±1.40^{a}
Overall score	97.17±1.25	80.00±1.65	88.50±2.31	88.17±2.59

Means within a row bearing different letters as superscript differ significantly.

Means within a row bearing same letters as superscript are homogenous.

The mean appearance and color, body and texture, flavor and overall scores of control paneer were 9.6±0.21, 39.17±0.40, 48.33±0.71and 97.17±1.25 respectively. The corresponding values for C2 were 6.83±0.4, 31.17±0.91, 42.00±1.51 and 80.00±1.65 respectively. The values of T1 paneer were 8.00±0.45, 34.33±1.38, 45.17±1.11 and 88.50±2.31 respectively. For l {T2 paneer the values were 8.33±0.33, 35.00±1.63, 45.83±1.40 and 88.17±2.59 respectively. The sensory scores were improved due to the incorporation of WPC. The body and texture of paneer added with WPC was superior to that of paneer prepared without WPC. This could be attributed to more softness in paneer due to the retention of moisture. Similar findings were also reported by earlier researchers. Narayanarao (2005) observed that the overall acceptability of low fat paneer incorporated with 2 per cent WPC was higher than control. Bhatt (2013) reported that the hardness values of WPC incorporated paneer samples were significantly lower than skim milk control paneer.

Physico-chemical quality of low fat paneer incorporated with inulin

Table 4 represents the physico-chemical analysis of low fat paneer incorporated with inulin

Table 4: Physico- chemical analysis of low fat paneer incorporated with inulin (Mean±S.E)

Parameters (%)	Full fat control (C1)	Skim milk control (C2)	Low fat paneer incorporated with 1.5% inulin (T1)	Low fat paneer incorporated with 2% inulin (T2)
Yield	17.90±0.48[a]	12.40±0.44[b]	12.62±0.41[b]	13.48±0.60[b]
Fat	19.50±0.34[a]	4.58±0.20[b]	4.25±0.21[b]	4.13±0.11[b]
Moisture	54.53±0.37[a]	57.26±0.23[b]	57.36±0.23[b]	59.28±0.29[c]
Total solids	45.47±0.37[a]	42.74±0.22[b]	42.64±0.23[b]	40.72±0.29[c]
Titratable acidity	0.48±0.48[a]	0.48±0.01[a]	0.48±0.01[a]	0.49±0.49[a]

Means within a row bearing different letters as superscript differ significantly.

Means within a row bearing same letters as superscript are homogenous.

Yield

The mean yield of control and treatment groups (C1, C2, T1 and T2) of paneer were 17.90±0.48, 12.40±0.44, 12.62±0.41and 13.48±0.60 per cent respectively. The yield of treatment groups of paneer and skim milk control paneer was significantly lower than full fat control paneer. Kantha (2005) also reported a linear and significant increase in the yield, with increasing levels of inulin in low fat paneer.

Fat

The mean fat percentage of control and treatment groups of paneer samples were 19.50±0.34 (C1), 4.58±0.20 (C2), 4.25±0.21 (T1), and 4.13±0.11 (T2) respectively. The fat per cent of treatment groups of paneer and skim milk control paneer were significantly lower than full fat control paneer. The decrease in fat content in inulin added samples might be due to the increase in moisture content caused by the water binding capacity of inulin. Similar findings were reported by the earlier researchers. Megha (2014) reported that the addition of inulin in goat milk yoghurt decreased the fat percentage. She also reported that the decrease in fat percentage was proportional to the level of addition of inulin. Teresa Grzelak (2016) reported that the addition of inulin to food products did not change their organoleptic features, but it allowed the reduction of fat content in the final product.

Moisture

The mean moisture content of paneer samples were 54.53±0.37, 57.26±0.23, 57.36±0.23 and 59.28±0.29 per cent respectively. There was a highly significant difference in moisture content between the treatment groups and full fat control paneer. Skim milk control paneer (C2) and treatment group one (T1) did not show any significant difference in moisture content; whereas, T2 showed significant variation from T1 and C2 Paneer samples prepared by incorporation of inulin had higher moisture content than skim milk paneer prepared without inulin. According to Kantha (2005), inulin had been reported to have the capacity to retain 1-2 gm moisture per each gram of inulin.

Total solids

The mean values of total solids content in paneer samples were 45.47±0.37 (C1), 42.74±0.22 (C2), 42.64±0.23 (T1) and 40.72±0.29 (T2) per cent respectively. The total solids content of treatment groups and skim milk control paneer showed significant difference from full fat control paneer. Addition of inulin had increased the moisture content and thereby reduced the total solids content.

Titratable acidity

The mean titratable acidity of control groups of paneer (C1 and C2) were 0.48±0.48and 0.48±0.01 per cent lactic acid respectively. The titratable acidity of treatment groups of paneer were 0.48±0.01and 0.49±0.49 per cent lactic acid for T1 and T2 respectively. The titratable acidity of treatment groups did not show any significant variation from control paneer. Kantha (2005) also found that there was no significant difference in titratable acidity between control and experimental samples incorporated with inulin.

Microbiological quality of paneer

Table 5 represents the microbiological quality of low fat paneer incorporated with inulin

Table 5: Microbiological quality of low fat paneer incorporated with inulin (Mean±S.E)

Microbiological analysis	Storage days	Full fat control (C1)	Skim milk control (C2)	Skim milk paneer incorporate with 1.5% inulin (T1)	Skim milk paneer incorporated with 2 % inulin (T2)
SPC	0th day	4.05± 0.01^{a}	3.98±0.05^{a}	3.98±0.04a	3.99±0.04^{a}
	4th day	4.14±0.03^{b}	4.11±0.033^{a}	4.08±0.02^{a}	4.09±0.03^{a}
	7th day	4.23±0.02^{c}	4.25±0.01^{c}	4.18± 0.02^{b}	4.20 ±0.02^{b}
Yeast and mold	0th day	1.26±0.09^{a}	1.26±0.09^{a}	1.26±0.09^{a}	1.18±0.08^{a}
	4th day	1.33±1.33^{a}	1.33±0.08^{ab}	1.39±0.09^{a}	1.48±0.06^{b}
	7th day	1.49±0.04^{a}	1.51±0.05^{b}	1.51±0.08^{a}	1.49±0.07^{b}
Coliform count	0th day	0.88± 0.18^{a}	0.72±0.23^{a}	0.22±0.22^{a}	0.72±0.23^{b}
	4th day	0	0.22±0.22^{a}	0	017±0.19^{ab}
	7th day	0	0	0	0

Means within a row bearing different letters as superscript differ significantly.

Means within a row bearing same letters as superscript are homogenous.

Standard plate count

The mean standard plate count of full fat control paneer was 4.04± 0.01, 4.14±0.03 and 4.23±0.02 log cfu/g respectively for 0th, 4th and 7thday of storage. The corresponding values of C2 paneer were 3.98±0.05, 4.11±0.03 and 4.25±0.01 log cfu/g respectively. The values of T1 paneer were 3.98±0.04, 4.07±0.02 and 4.18±0.02 log cfu/g respectively. For T2, the mean values were 3.99±0.04, 4.08±0.03 and 4.20±0.02 log cfu/g respectively. Standard plate count of paneer samples showed a significant increase during storage. However, all the samples of paneer met with the legal standards.

Yeast and Mould count

The mean yeast and mould count of full fat paneer were 1.26±0.09, 1.33±1.33 and 1.49±0.04 log cfu/g respectively for 0th, 4th and 7thday of storage. For C2 the counts were 1.26±0.09, 1.33±0.08 and 1.51±0.05 log cfu/g respectively. For treatment group one (T1), the mean values were 1.26±0.09, 1.39±0.09 and 1.51±0.08 log cfu/g respectively. In treatment group two (T2), the mean values were 1.18±0.08, 1.48±0.06 and 1.49±0.07 log cfu/g respectively. Yeast and mould count showed a progressive increase during storage. However, it was within the legal limit until seven days of storage. Yadav *et al.* (2009) had also reported an increase in yeast and mould count in paneer samples during storage.

Coliform count

The coliform count on 0th day was 0.88±0.18 log cfu/g and it was absent on 4th day of storage in full fat control (C1). For C2 the values were 0.72±0.23 and 0.22±0.22 log cfu/g on 0th and 4th day of storage respectively. For T1 the value was 0.22±0.22 log cfu/g on 0th day and it was absent on 4th day of storage. In treatment group two T2 the values were 0.72±0.23 and 0.33±0.21 log cfu/g respectively. Coliforms were absent in all paneer samples on 7th day of storage due to the increase in acidity of paneer during storage. Viji (2014) also reported that coliform counts were absent on 5th day of refrigerated storage in goat milk paneer samples. In the present study, coliform counts were within the acceptable limit prescribed by FSSA until seven days of refrigerated storage.

Sensory evaluation

Table 6 represents the sensory scores of low fat paneer incorporated with inulin.

Table 6 Sensory scores of inulin added low fat paneer(Mean ± S.E)

Parameters	Full fat control (C1)	Skim milk control (C2)	Skim milk paneer incorporate with 1.5% inulin (T1)	Skim milk paneer incorporated with 2 % inulin (T2)
Color and appearance	9.42±0.27^{a}	7.67±0.67^{b}	8.83±0.31^{ab}	8.75±0.31^{ab}
Body and texture	37.67±0.95^{a}	29.50±2.01^{b}	33.67±1.48^{ab}	31.50±1.26^{b}
Flavour	48.17±0.87^{a}	42.67±2.67^{a}	46.00±1.77^{a}	45.33±1.54^{a}
Overall score	95.25±1.61	79.83±3.61	88.50±2.88	85.58±2.48

Means within a row bearing different letters as superscript differ significantly.

Means within a row bearing same small letters as superscript are homogenous.

In inulin added paneer samples the mean appearance and colour, body and texture, flavor and overall scores of full fat control paneer were 9.42±0.27, 37.67±0.95, 48.17±0.87 and 95.25±1.61 respectively. The corresponding values for C2 were 7.67±0.67, 29.50±2.01, 42.67±2.67 and 79.83±3.61 respectively. The values for T1 paneer were 8.83±0.31, 33.67±1.48, 46.00±1.77and 88.50 ±2.88 respectively. For T2 paneer the values were 8.75±0.31, 31.50±1.26, 45.33±1.54 and 85.58±2.48 respectively. Kantha (2005) reported that paneer developed from milk with 1.8% fat and 4.5% inulin had similar sensory attributes to that prepared from full cream milk.

CONCLUSION

From the above results, it can be concluded that good quality low fat paneer could be prepared from skim milk by the addition of WPC or inulin. The yield and moisture content of paneer increased with the addition of WPC or inulin. The sensory scores of low fat paneer were improved due to the incorporation of WPC and inulin. The paneer prepared by the addition of 1.5 per cent inulin showed better sensory scores than 2 per cent inulin. The microbiological qualities of the developed low fat paneer samples were good until seven days of refrigerated storage. Low fat paneer prepared with the incorporation of 0.5 per cent WPC had maximum yield (15.73 per cent).

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Short Communications

Immunogenicity of pasteurella multocida cell associated and cell free antigens in mice

Sahzad, S. Arya, M. Bora, S. Shebannavar*, T. V. S. Rao and G. S. Reddy

Brilliant Bio Pharma Private Limited, I.D.A, Pashamylaram, Hyderabad - 502 307, Telangana, India

ABSTRACT

Haemorrhagic septicaemia is an economically important contagious disease of cattle, buffaloes and bison. In the present study, the efficacy of the vaccines prepared from cell free and cell associated antigens of disease causing organism *Pasteurella multocida* was evaluated in mice. From the present study, it was observed that both cell associated and cell free antigens provided protective immunity to the vaccinated animals in combination with each other and increasing the content of both the antigens improved the efficacy of the vaccine blends. In conclusion, use of both cell associated and cell free antigens in vaccine formulations are warranted for developing improved vaccines against Haemorrhagic septicaemia.

Key words: Haemorrhagic septicaemia, *Pasteurella multocida,* Cattle, Vaccines, Cell free antigen, Cell associated antigen.

Haemorrhagic septicaemia (HS) is an important fatal and contagious disease of cattle and buffaloes. It is caused by *Pasteurella multocida*, a Gram negative bipolar bacterium. The organism causes pathological changes in the respiratory tract leading to state of generalized septicaemic condition. The affected animal often leads to death in absence of timely treatment with antimicrobials. Due to its contagious nature, the disease can lead to economic devastation in the lives of farmers. In some parts of India alone, the disease is estimated to have caused losses to the tune of USD 1,30,000 between 2007 and 2011 (Singh *et al*., 2014).

Vaccination of susceptible hosts has been the most successful strategy for control of haemorrhagic septicaemia outbreaks. Inactivated vaccines are most commonly used for the purpose and include cell associated and cell free bacterial antigens along with one of the commonly used adjuvants such as alum, aluminium hydroxide and mineral oil. The oil adjuvanted vaccine provides high degree and duration of immunity compared to other adjuvanted vaccines. However, it suffers from the disadvantage of having high viscosity and poor syringibility

***Corresponding author**
Dr Sunil Shebannavar
Brilliant Bio Pharma Pvt Ltd
Plot No 97, 98, 276 & 277, I.D.A.,
Pashamylaram, Sangareddy (Dist)-502307
Telangana, India
Email: sunil.s@bbpl.co.in

during administration in the field (Saad and Anna, 2016). Therefore, present study was undertaken to evaluate the usefulness of aluminium hydroxide adjuvant with cell associated antigen and cell free antigens of *Pasteurella multocida.*

The *Pasteurella multocida* (P-52) vaccine strain procured from Indian Veterinary Research Institute, Izatnagar was passaged in healthy rabbit and the infected blood was stored in freeze dried form at 2-8°C. Further, the rabbit passaged freeze dried vaccine seed was used for preparation of vaccines with varying antigen payloads. Briefly, one vial of freeze dried rabbit passaged HS seed was inoculated into 200 ml of HS media and incubated at 37°C overnight under constant stirring. The 200 ml of HS vaccine strain seed culture was further inoculated into 10 L of HS media and incubated at 37°C overnight under stirring. The culture was inactivated by adding formalin to final concentration of 0.5% and incubating under stirring at 37°C for 24 h. The inactivated harvest was centrifuged at 6000 rpm for 45 min and, the supernatant and pellet were separated. The cell pellet was resuspended in 200 ml of 0.2% formal saline and again centrifuged at 6000 rpm and for 45 min. Subsequently, the cell pellet was resuspended in 180 ml of 0.2% formal saline and stored at 2-8°C until used for vaccine preparation. The 9.5 litre culture supernatant was concentrated to 460 ml by using synthetic hemodialyzer (ELISIO™ -13M, NIPPRO). Around 80 ml of pellet and concentrated culture supernatant was stored separately as source of cell associated and cell free antigens of *P. multocida*, respectively, while preparing vaccine blends. A total of four vaccine blends containing aluminium hydroxide as adjuvant were prepared namely; Blend-I: 1 ml culture equivalent of both cell associated antigen and cell free antigen, Blend-II: 2 ml culture equivalent of both cell associated antigen and cell free antigen, Blend-III: 1 ml culture equivalent of cell free antigen and Blend-IV: 1 ml culture equivalent of cell associated antigen (Table). All the four vaccine blends were tested for potency in mice by challenge with virulent *P. multocida* as per Indian Pharmacopoeia (2018) with prior approval from Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). The lethal dose 50 (LD_{50}) for each of the vaccine blends including unvaccinated controls were calculated by using Reed and Muench (1938). The titre of virulent *P. multocida* as observed in group of control mice was $10^{7.5}$ LD_{50}. The Protective Index (PI) was calculated as below.

PI = LD_{50} in control mice ÷ LD_{50} in vaccinated mice

LD_{50} = Reciprocal of 50% endpoint dilution.

Log_{10} 50% end point dilution = Log_{10} of dilution showing a mortality next above 50% - (Proportionate distance x logarithm of dilution factor).

Proportionate distance = [(mortality at dilution next above 50%) – 50%] / [(mortality next above 50%) – (mortality next below 50%)]

Table 1 Composition of vaccine preparations with cell associated antigens and cell free antigens of *Pasteurella multocida*

Blend	Antigen type	Culture volume equivalent/ dose	Antigen volume/dose	Aluminium hydroxide / dose	0.2% formal saline / dose	Potency (Protection Index) Log_{10} values
I	Cell associated + Cell free	1 ml	0.1 ml	0.9 ml	1 ml	4.1
II	Cell associated + Cell free	2 ml	0.2 ml	0.9 ml	0.9 ml	4.5
III	Cell free	1 ml	0.1 ml	0.9 ml	1 ml	3.5
IV	Cell associated	1 ml	0.1 ml	0.9 ml	1 ml	3.7

The calculated PIs for Blend-I containing 1 ml culture equivalent of cell associated and cell free antigen /dose was 4.1, Blend-II containing 2 ml culture volume equivalent/dose containing both cell supernatant and cell pellet was 4.5, Blend-III containing 2 ml culture volume equivalent of cell supernatant was 3.5 and Blend-IV containing 1 ml culture volume equivalent/dose of cell associated antigen was 3.7. A close analysis of results reveal that the blends having combination of both cell associated and cell free antigen provided better protection to virulent *P. multocida* challenge in mice but no protection when administered alone. Increasing the antigen content of both the antigens (2 ml culture equivalent) yielded marginally better protection compared to 1 ml culture volume equivalent of antigens. Various factors such as outer membrane proteins (OMP), lipopolysaccharides, secreted bacterial toxins and stress induced proteins have been identified to play an important role in the pathogenesis and host cell evasion of *P. multocida* (Ghani *et al*., 2016). These factors also act as targets for host immune responses leading to development of protective immunity against the infecting organisms. Recently, Uchida *et al*., (2003) showed higher protective indices and prompt clearance of toxigenic strains of *P. multocida* in mice immunized with inactivated cell free antigen than purified and inactivated *P. multocida* toxin. Similarly, 100% protection in mice immunized with cell associated antigen (OMP) has also been reported (Joshi *et al.,* 2013). In conclusion, present study showed that both cell associated antigens (such as OMP and capsular antigens) and cell free antigens (toxins and stress proteins) elicit immune responses and the protective immunity is enhanced by combining both the antigens in the vaccine formulations.

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Occurrence of proventriculo-ventricular intussusception in chicken - A one-year perspective study

M. Pradeep* **and M.R. Reddy**
Department of Veterinary Pathology, College of Veterinary and Animal Sciences, Pookode, Wayanad, Kerala, India

Abstract

Proventriculo-ventricular intussusception is the telescoping of proventricular portion of the avian stomach into the ventriculus. Even though occurrence of intestinal intussusceptions in chickens commonly observed, the reports on proventriculo-ventricular intussusceptions were very scarce. The study was done as a part of screening the gut lesions in the chicken carcasses for a period of one year and 18078 chicken carcasses of multiple age groups belonging to 15 pure line breeds and two commercial breeds were screened. Proventriculo-ventricular intussusceptions noticed in two female PD2/Vanaraja chicks of 4 days and 3 weeks of age and in a 9 weeks old male Nicobari grower. While the intussuscepted proventriculus of Vanraraja chicks had unremarkable inflammatory lesions, severe congestion of proventriculus along with koilin displacement in the anterior portions of ventriculus were evident in Nicobari grower. The present study, point out the occurrence of proventriculo-ventricular intussusception in young synthetic and native lines of chicken.

Key words: Proventriculo-ventricular intussusception, Vanaraja, Nicobari breed.

Intussusception is the telescoping of one part of the digestive tube into the lumen of adjacent part often resulting in blockage of feed and fluid passage. The inner telescoped part is called the intussusceptum and the outer receiving part intussuscepiens. Even though the intussusception of intestine is rather common in poultry (Crespo *et al*., 2013), intussusception involving proventriculus scarcely been reported (Shrivastava *et al.*, 1989). Because of the scarcity of reports, its incidence and aetiological studies are not available. The present study aimed to throw light on the occurrence of proventriculo-ventricular intussusception in chickens of different breeds and age groups.

The study was based on the necropsy screening of chicken carcasses for gut lesions for a period of one year spanning from December 2015 to November 2016. Necropsy was performed on daily basis on the carcasses from the farms of ICAR- Directorate of Poultry Research, Hyderabad, Telangana, India and on the occasional outbreak based necropsy from the commercial farms. A total of 18078 carcasses belonging to 15 different breeds/lines and multiple age groups were screened during the period. The lines screened included white Leghorn breeder

*Corresponding author: M. Pradeep
email: pradeep.mampilli@gmail.com

lines such as IWA, IWD, IWF IWH, IWI, IWK (n= 2095), synthetic breeder lines like PD1 (n=1712), PD2/Vanaraja (n=3880), PD3 (n=4223), Gramapriya male line (n=1632), broiler lines like Punjab Broilers (n=2783), single gene lines like Dwarf (n=237) and Naked Neck (n=257), and native breeds like Aseel (n=341), Ghagus (n=392) and Nicobari (n=426). Apart from this pure lines, commercial White Leghorns (n=52) and commercial broiler breeders (n=48) were also screened during the period. Tissue samples were collected in 10% neutral buffered formalin processed by paraffin embedding method, sectioned at 5µ thickness and stained with routine Haematoxylin and Eosin (H&E) stain.

The occurrence of Proventriculo-ventricular intussusceptions was 0.017% (3/18078). The condition noticed in two female PD2/Vanaraja (0.052%) birds of age, 4 days and 3 weeks, and in one male Nicobari (0.236%) aged 9 weeks. All the three affected chickens were floor reared and belonged to different flocks reared in different sheds. In these chickens, the proventriculus was telescoped into the lumen of anterior thin walled part of the ventriculus. Closer observation revealed that the point of telescoping originated posterior to the proventriculo- oesophageal junction without the eversion of the oesophagus. The intact oesophagus was drawn posteriorly along with the telescoped proventriculus. In the Vanaraja chicks (Fig. 1), very small part of the inverted proventriculus was protruded into the lumen of ventriculus while moderately protruded in Nicobari grower.

Figure 1 Proventriculo-ventricular intussusception (arrow) in the 4 day old Vanaraja chick

Inflammatory lesions were absent in the proventriculus and ventriculus of both Vanaraja chicks. Pulling of proventriculus with mild force, holding the oesophagus relieved the intussusception in the 3 weeks old Vanaraja chick. Histologically mild degeneration and compression of the submucosal glands were found in the Vanarja chicks (Fig. 2). On the other hand, the intussusception was not relieved with mild pulling of the oesophagus in case of Nicobari grower. Further, the koilin layer of the ventriculus was pealed posterior to the isthmus and pushed into the lumen by the protruding proventriculus (Fig. 3). The intussusceptum was severely congested (Fig. 4). Crop was empty in all the three cases.

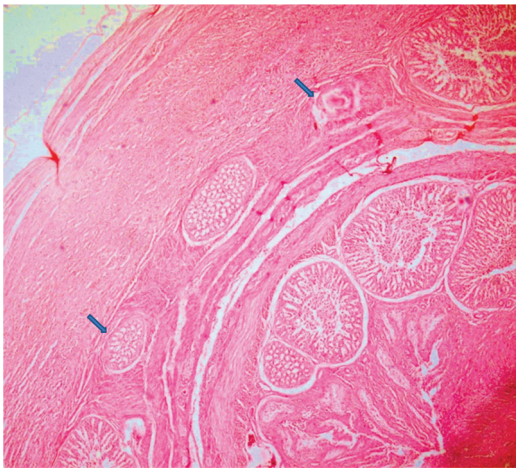

Figure 2 Degeneration and compression of proventricular glands (arrow) in the proventriculo-ventricular intussusception. H&E 40x

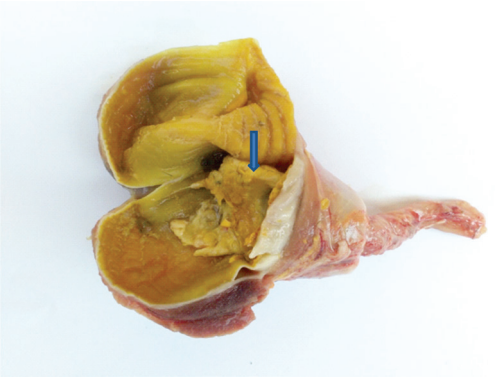

Figure 3 Proventriculo-ventricular intussusception in the Nicobari grower. Koilin layer can be seen pushed into the lumen of the ventriculus (arrow)

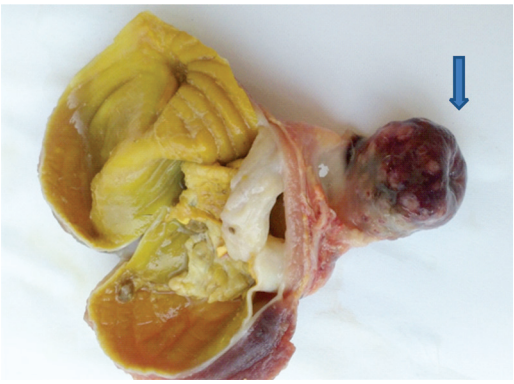

Figure 4 Severe congestion of the intussusceptum proventriculus (arrow) of Nicobari grower

Gross lesions were not observed in the intestines of any of the cases. Coccidial oocysts were randomly noted in the caecal contents of 3 weeks old Vanaraja chicks but not in other birds.

The present study showed that proventriculo-ventricular intussusception even though rare, occurred in young chicken. PD2/Vanaraja is a dual purpose breed developed for backyard rearing with medium growth rate while Nicobari is a native breed with its origin in Andaman and Nicobar islands of India with smaller size and slow growth rate. This indicates that the growth rate may not have significant contribution in the development of the condition. Further, broiler breeds with high growth rate has not affected in the study.

Inflammatory reactions were absent in the telescoped proventriculus in the previous report (Shrivastava *et al*., 1989) and in both Vanaraja chicks of the present study. This may be due to the acute development of the condition. However, the lesions in the Nicobari were different from the early report with severe vascular changes in the telescoped proventriculus along with pealing of koilin layer of ventriculus. Absence of fibrous tissue adhesions indicates a subacute nature of the condition.

Studies on aetio- pathogenesis of proventriculo-ventricular intussusception are not available in the literatures. The aetiology of commonly occurring intestinal intussusceptions are also obscure. In human, intestinal intussusceptions are very common in infants (WHO, 2002) and probable aetiology like adenovirus and enterovirus (O'Ryan *et al*., 2003) and some other infectious, neoplastic and functional disturbances were hypothesized (Cera, 2008), actual aetiology could not be ascertained so far. On the animal side, intestinal intussusception had been found along with parasitism (Wilson and Burt, 1974), foreign bodies, viral enteritis, intestinal surgeries and neoplasia (Levien and Baines, 2011). In fowls, multiple predisposing conditions like coccidiosis, mucosal injury and intestinal hyper motility were considered as the possible causes of the intestinal intussusception (Williams,

1986). In the present study, coccidial oocysts found only in one case but that too was very insignificant in severity. Hence, coccidiosis and resultant hypermotility will not be the cause of provntriculo-ventricular intussusception. A close follow up with other chicken of the same flocks showed no recurrence of the condition, that excludes possibility of any infectious cause. Chronic dyspepsia, chronic inspiratory difficulty due to upper airway obstruction and increased abdominal pressure were attributed for the development of gastro-oesophageal intussusception in dogs (McGill *et al*., 2009). Starvation considered as a probable cause of intestinal intussusception in fowls by some workers (Okoye, 1985) but ruled out by others (Williams, 1986). Although, empty crops observed in all the three birds, role of hunger in the development of provntriculo-ventricular intussusception require further study.

The present study revealed rare occurrence of proventriculo-ventricular intussusception in young chickens of dual purpose lines as well as native breeds. Unlike the earlier reports, proventriculus with severe congestion observed in the present study. Actual cause for the development of the proventriculo-ventricular intussusception is unclear. The possibility of occurrence of proventriculo-ventricular intussusception need to be considered while making clinical diagnosis especially in well-priced birds.

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