Effect of humectants on physico-chemical and sensory scores of hurdle technology-based shelf stable chicken pickle during storage period

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Abstract
This study was designed to evaluate the effect of lactic acid as acidulants and glycerol, sorbitol, and honey as humectants on the storage stability of shelf-stable chicken pickles. Chicken pickle was prepared according to method prescribed by Das et al. (2013) [3] with slight modifications. An attempt was made to improve the shelf life of steam-cooked chicken pickles by incorporating acidulant i.e. lactic acid at 1% level and humectants i.e. glycerol at 3% level (GY) or honey at 6% level (HY) or sorbitol at 6% level (SB). Control and treated chicken pickles were well acceptable up to the 60th day of storage based on sensory score and physico-chemical properties. The pH, TBARS, and FFA values of HY, GL, and SB were significantly (p<0.05) lower whereas titrable acidity was significantly (p<0.05) higher than the control during storage. The values of titrable acidity, TABRS, and FFA value of control as well as treatments increased significantly (p<0.05) whereas pH and sensory scores decreased significantly (p<0.05). Among treatments, HY had higher oxidation stability along with significantly (p<0.05) higher overall acceptability scores. This study concluded that the more stable chicken pickle could be prepared by incorporating 1% lactic acid and 6% honey addition.

Keywords: Sensory evaluation, humectants, shelf stable, hurdle technology, acidulants, chicken pickle

Introduction
Traditional food enriches our sensory perception by providing a wide variety of flavour, colour, and texture. Pickling is an age-old traditional method for the preservation of food. Pickle is well known for its appetite-enhancing properties and helps in the digestion of food by stimulating the flow of gastric juices. Meat pickles are traditionally unfermented, delicious, spicy ready-to-eat and nutritious meat preparation generally stable for several weeks due to low moisture content (Maiti et al. 2009) [23]. Pickling helps to utilize tough low-quality meat and process it into shelf-stable convenience products. Chicken meat pickle is a shelf-stable intermediate moisture type product. It is value added convenient product containing various ingredients like meat, spices, condiments, oil, vinegar, and other food additives. Cooking and frying during pickle making denatures meat proteins and decreases the water-holding capacity of meat resulting in loss of water and increasing its shelf life. However, the quality characteristics of chicken pickles are also dependent on the characteristics of meat, formulation, and processing technique as well as time/temperature evolution during cooking.

The pH of the food also significantly impacts the lethality of heat treatment of the food. The pH value below 5.0 is considered critical for storage stability of meat products (Dziezak 1986) [6]. Higher acidity, salt content, cooking, frying, and low moisture reduces the microbial load of the product. Therefore, acidification of meat pickles is intended to prevent the growth of microorganisms and make the product shelf stable at room temperature. Acidified products may limit microbial growth or survival depending on the types of microorganisms harboured in the food and the type and amount of acid used. Fermentation and the addition of organic acids have been used as a preservation method since ancient times to increase the acidity of foods. Organic acids are more effective preservatives in an undissociated state. Lowering the pH of a food increases the effectiveness of an organic acid as a preservative.
An important phenomenon that is crucial to hurdle technology is the homeostasis of microorganisms. Homeostasis is the constant tendency of microorganisms to maintain a stable and balanced (uniform) internal environment. Preservative factors functioning as hurdles can disturb one or more of the homeostasis mechanisms, thereby preventing microorganisms from multiplying and causing them to remain inactive or even die. Some of the hurdles such as temperature (high or low), water activity, preservatives (nitrite, sorbate), competitive microorganisms (lactic acid bacteria), and acidity (pH) have been empirically used for years to stabilize meat, fish, milk, and vegetables (Leistner 2000) [15]. Reduced water activity and pH are the two major hurdles contributing to the shelf stability of meat pickles. In meat, moisture is quantitatively the most important component of the product, constituting up to 75% by weight.

The amount of water in meat products is also directly related to its water holding capacity as well as water binding capacity. Water in meat is associated with muscle tissue and protein has a central role in the mechanism of water binding. The basic principle for the preservation of meat products via moisture control can be achieved in two ways: either by reduction of moisture content (freeze-drying, dehydration, smoking, etc.) or by reducing water activity i.e. water is present in the product, but not available for the growth of microorganisms. The best way to reduce water activity in meat products is the addition of humectants. Since humectants are substances that attract water to themselves, they can retain water in foodstuffs, reduce water activity, and perform the important function of improving food softness. The addition of humectants such as glycerol, sorbitol, honey, etc., in meat pickles may achieve water activity reduction along with pickle storage stability and reduction in microbial count. Glycerol is a water-soluble, odourless, colourless, naturally occurring humectant commonly used in the food and cosmetic industry. It has a caloric density similar to table sugar, but a lower glycaemic index and different metabolic pathways within the body, so some dietary advocates also accept glycerol as a sweetener compatible with low-carbohydrate diets. Sorbitol is a sugar alcohol with a sweet taste obtained by the reduction of glucose, which changes the aldehyde group to a hydroxyl group. It is highly soluble in water and is an excellent humectant. Sorbitol is about 60% as sweet as sucrose with fewer calories. It has a smooth mouthfeel with a sweet, cool, and pleasant taste. Sorbitol also protects the soft and moist texture of food when added as a 70% solution. Honey is the only natural humectant in a concentrated sugar form that is used for food preservation worldwide with a great ability to hold onto water (FAO 2015) [9]. Honey has a pH balance of between 3 and 4.5, is highly acidic, and contains hydrogen peroxide. Honey also provides antioxidant effects against the deterioration of food due to light, heat, and metals.

Material and Methods

The experiments were conducted in the Department of Livestock Products Technology, College of Veterinary Science and Animal Husbandry, DUVASU, Mathura. Live spent poultry birds were procured from the Department of Poultry Science, DUVASU, Mathura. These birds were taken, given rest for 1-2 hours, and then slaughtered following the standard procedure (Halal method). The lean carcass was eviscerated and dressed carcass was kept for conditioning in a refrigerator at 4±1°C for 4-6 hours and then frozen at -18 °C till further processing. All other ingredients like salt, mustard oil, vinegar, spices of Agmark grade, condiments, etc. required for product preparation were procured from the local market of Mathura. All the chemicals used in the study were procured from Hi Media Laboratories (P) Ltd, Mumbai, India. Thermo rigid air-tight PET containers were sourced from the local market for packaging and were pre-sterilized by exposing them to UV light for 30 minutes before use.

Preparation of chicken pickle

The chicken pickle was prepared using Das et al. (2013) [3] method with slight modifications (Singh et al. 2019) [22]. Thawed chicken meat were cut into 1-2 inch chunks, and marinated with 1% salt and 1% turmeric powder for 30 minutes. The marinated chicken meat was then steam cooked (without pressure) for 15 minutes and then fried at 175±5°C in pre-warmed mustard oil to get golden brown colour. In a separate kadahi containing prewarmed oil (500 ml), mustard seeds, condiments (8 gm), spice mix (3 gm), and salt (3 gm) were added consecutively followed by the addition of fried meat chucks and white vinegar (10 ml) and cooked for 5 minutes. The pickle was cooled to room temperature and packed in a pre-sterilized air-tight PET container, with the remaining heated mustard oil filled to the top without leaving any air space. Chicken pickle was left for the next 2 days at ambient temperature for maturing and then used for further analysis.

Physico-chemical properties

pH

The pH was determined by using a digital pH meter (WTW, Germany, model pH 330i) by immersing the spear-type combination electrode (Sentix®, Germany) directly into the minced meat sample following the procedure of Trout et al. (1992) [35]. Reading was taken twice for each sample and the average of the reading was taken as the pH of the sample.

Titratable acidity

The titratable acidity of chicken pickle was determined by homogenizing 10 g of each sample with 100 ml distilled water. About 10 ml aliquots were titrated against 0.01N NaOH in the burette using 0.1% phenolphthalein solution as an indicator. The volume of 0.01N NaOH per g of the sample utilized was expressed as titratable acidity.

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\text{Titratable acidity} \% = \frac{\text{Sample weight (g) x Volume used x eq. wt. of acid}}{\text{Titre x Normality of NaOH solution x Volume used x eq. wt. of acid}} \times \frac{1}{10}
\]

Thiobarbituric acid reactive substances (TBARS)

TBARS value was estimated as per the procedure given by Tarladgis et al. (1960) [34]. 10 g of sample was taken and added to 49 ml of distilled water and 1 ml of sulphanalimide reagent (1 gram of sulphanalimide dissolved in solution containing 40 ml of conc. HCl and 160 ml of distilled water) and blended with the help of pestle and mortar. After this 48 ml of distilled water was used for washing the mortar and to it 2 ml HCl solution (diluted 1:2 with distilled water) was added. The contents were transferred to the Kjeldahl flask after adding several glass beads. This was heated on high heat and 50 ml of distillate was collected into a graduated cylinder. After mixing the distillate well, 5 ml portion was taken into a 50 ml glass stoppered flask, and 5 ml of TBA...
**reagent (0.02 M 2-thiobarbituric acid in 90% glacial acetic acid) was added. The contents were mixed and the flask was immersed in a boiling water bath for exactly 35 minutes along with blank solution. The blank prepared was consisting of 5 ml distilled water and 5 ml of TBA reagent. The flasks were then cooled under tap water for 10 minutes and the optical density (O.D.) was recorded at 538 nm against blank. The TBARS value as mg of malonaldehyde per Kg of the sample was calculated using the following formula:

$$\text{TBARS value (mg of malonaldehyde/Kg of the sample)} = \frac{0.1 \text{ ml of 0.1 N alcoholic KOH used in titration} \times 0.282}{\text{Sample weight (g)}} \times 100$$

**Sensory evaluation**

The sensory quality of samples was evaluated by using an 8-point hedonic scale (Keeton et al., 1983) where 1=extremely disliked and 8= extremely liked. A sensory panel (semi-trained) of seven judges drawn from postgraduate students and faculty of Veterinary College, DUVASU, Mathura were requested to evaluate the product for different quality attributes viz., colour and appearance, flavour, texture, juiciness, saltiness, sourness, and overall acceptability.

**Statistical analysis**

Duplicate samples were taken and three trials were conducted for each physico-chemical parameter, a total being six observations (n=6) and Seven samples were taken and three trials were conducted for each sensory score, a total being twenty-one observations (n=21) for consistency of the results. The data generated from various trials under each experiment were pooled and analyzed by the statistical method of one way- ANOVA and mean ± S.E. using Statistical Package for the Social Sciences - 16.0 software package developed as per the procedure of Snedecor and Cochram (1995) [23] and calculated means were compared by using Dunkan’s multiple range test at 5% level of significance (Dunkan 1955) [7]. Statistically analyzed data was tabulated and interpreted.

**Result and Discussion**

Hurdle technology-based shelf stable chicken pickle incorporated with optimum level of acidulant i.e. 1% lactic acid and humectants i.e. 3% glycerol or 6% honey or 6% sorbitol in treatments along with control were stored at ambient temperature (25±2°C) and evaluated for their storage stability at every 10th day interval till two months or incipient spoilage was evident. Picture of control and hurdle technology based shelf stable chicken pickle on 0 day, 30th day and 60th day are presented in figure 1.

**Physico-chemical properties**

**pH**

The pH is an important intrinsic factor that influences the growth of microbes in meat and meat products (ICMSF 1980) [13]. The mean pH values of humectants incorporated treatments were significantly (p<0.05) lower than control on all storage days due to the incorporation of lactic acid and humectants viz. glycerol, sorbitol, and honey in chicken pickle. SB had significantly (p<0.05) lower pH than GL and HY throughout the storage period. The possible reason behind this finding might be the incorporation of sorbitol at 6.0% level which is slightly more acidic than glycerol at 3.0% level and honey at 6.0% level in product. There was no significant difference between GL and HY on the 0 and 10th day, however, the pH of HY was significantly (p<0.05) lower than GL from the 20th to 60th day. The pH of control, as well as treatments, decreased significantly (p<0.05) with the progression of storage, although the rate of decrease in pH was slower in the case of acidulant and humectant-treated products. The decrease in the pH of chicken pickles during storage might be due to the activity of certain types of fermentative microorganisms, which may produce acid resulting in a lowering of the pH of meat products. Gupta and Basu (1985) [10] also observed a decreasing trend in pH values during the storage of cockle meat and in fish pickles prepared with vinegar. Wani and Majeed (2014) [36] also reported significantly (p<0.05) lower pH of gizzard pickles during storage from 0 to 60 days at ambient temperature.

**Titrable acidity**

Titrable acidity is an indication of the pH and microbial quality of acidic meat products. Titrable acidity of control and treated pickles are presented in figure 2. Treatments had significantly (p<0.05) higher titrable acidity than control throughout the storage period due to the incorporation of lactic acid and humectants viz. honey sorbitol and glycerol respectively in treatments. These findings could be correlated with the results of pH observed in chicken pickles during storage in the present study. Wani and Majeed (2014) [36] also observed higher titrable acidity of gizzard treated with organic acid than control due to the absorption of acetic acid into the gizzard muscle through capillary forces by pressure gradient exerted by internal deformation of the meat. SB had significantly (p<0.05) higher titrable acidity than GL followed by HY on each day of storage study. The titrable acidity of control, as well as treatments, increased significantly (p<0.05) with the progression of storage. Pal and Agnihotri (1994) [27] observed no significant change in the titrable acidity of meat pickles up to the 45th day, however, increased significantly (p<0.05) with further progression of storage up to the 60th day due to the growth of fermentative microorganisms.

**Thiobarbituric acid reactive substances (TBARS)**

TBARS number is a measure of secondary lipid oxidation products like malonaldehyde, which is formed during the oxidation of fat in meat (Olsen et al. 2005) [26]. TBARS value of control and treated pickles are presented in figure 3. The mean TBARS values of HY, SB, and GL were significantly (p<0.05) lower than control on all storage days due to antioxidant and antimicrobial properties of lactic acid.
as well as humectants viz. glycerol, sorbitol, and honey added in treatments. There was no significant difference among the treatments up to the 20th day; thereafter HY had significantly (p<0.05) lower TBARS values than GL and SB from 30th to 60th day. Lower TBARS values in HY than GL and SB might be due to the antioxidant properties of honey added at a 6% level in HY. Khalil et al. (2010) reported that the superoxide radical scavenging activity of honey was due to the presence of vitamin C, phenol compounds, catalase, peroxides, and glucose oxidase enzymes. The mean TBARS values of control, as well as treatments, increased significantly with the progression of storage because of increased lipid oxidation and production of volatile metabolites in the presence of oxygen. However, TBARS value did not cross the acceptable level of 1-2 mg malonaldehyde/kg meat for spoiled meat (Witte et al. 1970) in control as well as treatments. Similar findings were observed by Sachdev et al. (1994) and Rout et al. (2015) in chicken gizzard pickles and chicken pickles incorporated with byproducts respectively.

Free fatty acids (FFA)
FFA content is a measure of hydrolytic rancidity in meat products determining the quality of the product and expressed as % oleic acid. The mean FFA values of control and treated pickles are presented in figure 4. The mean FFA values of HY, SB, and GL were significantly (p<0.05) lower than the control on all storage days due to the presence of organic acid and humectants which provided oxidative stability of the product. HY had significantly (p<0.05) lower FFA values than SB from 0 to 20th day, whereas values of GL were comparable to HY and SB. Thereafter, HY had significantly (p<0.05) lower values than GL and SB with the progression of storage from 30th to 60th day. Lower FFA values in HY were due to the presence of various phytochemicals and phenolic compounds in honey exhibiting antioxidant and antimicrobial properties (Bertoncelj et al. 2007) [1]. The mean FFA values of control, as well as treatments, increased significantly (p<0.05) with the progression of storage. The increase in FFA values with the advancement of storage time might be due to fat oxidation, enzymatic or microbial lipolysis, and its conversion into free fatty acid. Similar increases in FFA content during storage were also reported by Nagamallika et al. (2006) [25] in chicken patties, Das et al. (2008) [26] in goat meat patties, and Kumar et al. (2011) [19] in chicken nuggets.

Sensory Evaluation
Colour and appearance
Comparison of sensory score of control and treatments are presented in figure 5. There was no significant difference between the control and treatments up to the 30th day, thereafter control had significantly (p<0.05) lower scores than treatments from 40th to 60th day. It could be due to a higher rate of lipid oxidation and non-enzymatic browning of control than treatments during storage. GL had significantly lower scores than HY and SB from 40th to 60th day due to the comparatively dark colour product as also supported by findings in the present study. Colour and appearance scores of control, as well as treatments, decreased significantly (p<0.05) with the progression of storage. The decrease in colour and appearance scores at a later stage of storage might be due to the rapid oxidation of myoglobin and lipid resulting in non-enzymatic browning.

Hamzeh et al. (2012) [11] also reported decreased colour scores in uncoated fish fillets during storage. Kumar and Sharma (2004) [21] also reported a decrease in general appearance scores in aerobically packaged low-fat pork patties stored under refrigeration temperature. A decrease in general appearance score with the progress of the storage period was also reported by Kumar et al. (2007) [20] and Das et al. (2006) [4] in chicken spread and comminuted meat respectively.

Flavour
Flavour is a complex sensory attribute that involves both taste and odour of the product. The flavour scores of C and HY were significantly (p<0.05) higher than SB and GL on the 0, 10th, and 20th day, thereafter scores of C were significantly (p<0.05) lower than treatments up to 60th day due to a higher rate of lipid oxidation and microbial growth as compared to treatments. Among the treatments, HY had significantly (p<0.05) higher flavour scores than SB and GL throughout the storage period as HY was much liked by sensory panelists due to the slight sweetish-sour flavour and pleasant aroma provided by honey. The flavour scores decreased significantly (p<0.05) in control as well as treatments with the progression of storage period due to oxidation of fat, liberation of fatty acids, and increased microbial load (Sahoo and Ajaneyulu 1997) [30]. Shukla and Srivastava (1999) [32] also reported a significant (p<0.05) decrease in flavour scores of chicken pickles with the progression of storage period. The progressive decrease in flavour scores could be correlated to an increase in TBARS values and free fatty acids in meat products under aerobic conditions (Tarladgis et al. 1960) [34].

Texture
Texture scores of C and HY were significantly (p<0.05) higher than GL and SB on 0 and 10th days, thereafter HY had significantly (p<0.05) higher scores than C, GL, and SB till the end of the storage due to antioxidant and antimicrobial properties of honey resulting into a slower rate of lipid oxidation and microbial growth maintaining the desirable texture of product for a longer period. There was no significant difference between C, GL, and SB from 20th to 40th day; but C had significantly (p<0.05) lower scores than treatments on 50th and 60th day due to a rapid increase in fermentable bacterial load with the advancement of the storage period. The texture scores of control and treatments decreased significantly (p<0.05) with further storage of the product due to relative reduction of juiciness and gradual loss of moisture that led to hardening of the product. Sharma and Nanda (2002) [31] also observed a progressive but slow decrease in crispiness of chicken chips packed in nitrogen atmosphere during storage for a period of 12 weeks at ambient temperature. The decreasing trend in texture scores in chicken meat nuggets were also reported by Yavas and Bilgin (2010) [34] during storage under refrigeration temperature.

Juiciness
Juiciness scores of HY were significantly (p<0.05) higher than control and other treatments throughout the storage period however there was no significant difference between C, GL, and SB. Higher juiciness scores of HY might be due to softness and tenderness provided by honey to the product. Endy and Lee (2016) [8] also observed higher tenderness
scores of duck jerky incorporated with honey than rice syrup and sorbitol as humectants. The mean juiciness scores of control, as well as treatments, decreased significantly \((p<0.05)\) with progression of storage. The decrease in juiciness scores could be attributed to loss of moisture from the surface of products during storage (Bhat et al. 2011) \([2]\), Ibrahim et al. (2012) \([12]\) also reported a significant \((p<0.05)\) decrease in juiciness score in beef patties during storage under refrigeration.

**Saltiness**
There was no significant difference between control and treatments up to 20th day, after that HY had significantly \((p<0.05)\) higher scores than C, GL, and SB from 30th to 60th day due to higher juiciness and less surface dehydration of honey incorporated chicken pickle. The scores decreased significantly \((p<0.05)\) in all the products with progression of storage period. The C, GL, and SB had no significant difference up to 10th day, where saltiness scores of HY had significant change up to 30th day. However, decreased significantly \((p<0.05)\) with the progression of storage period. Khanam (2017) \([17]\) also observed no significant change in saltiness scores of chicken spread up to 20th day, but scores decreased significantly \((p<0.05)\) with further storage of product.

**Sourness**
The sourness is one of the important factors for acceptability of meat pickle. Sourness scores of HY were significantly \((p<0.05)\) higher than control and other treatments throughout the storage period due to desirable sweetish-sour flavour which was much liked by sensory panelists. SB had significantly \((p<0.05)\) higher scores than C and GL. However, there was no significant difference between C and GL from 0 to 60th day. Lower scores of C and GL were due to no addition of any organic acid in C and tasteless nature of glycerol added in GL respectively. However, sorbitol and honey provided sourness and sweetish-sour taste to the product. The sourness scores of control, as well as treatments, decreased significantly \((p<0.05)\) with progression of storage. The possible reason behind these findings might be the production of acid due to growth of fermentable microorganisms resulting into lowering of \(\text{pH}\) of chicken pickle.

**Overall acceptability**
It refers to the sensory perception of a meat product in totality in absolute terms. C and HY had significantly \((p<0.05)\) higher scores than GL and SB on 0 and 10th day. Thereafter, HY had significantly \((p<0.05)\) higher scores than C, GL and SB till end of the storage due to desirable flavour, taste, and texture of product maintained till the end of the storage. Endy and Lee (2016) \([8]\) prepared restructured duck jerky with incorporation of different humectants and reported that product prepared with incorporation of 10% honey had highest sensory scores. Mokhtar et al. (2014) \([24]\) also observed significantly \((p<0.05)\) higher odour, colour, taste and overall acceptability scores for antioxidants treated beef patties than control. There was no significant difference between C, GL, and SB from 20th to 40th day, then C had significantly \((p<0.05)\) lower scores than treatments on 50th and 60th day rapid increase in fermentable bacterial load with the advancement of storage period. The overall acceptability scores of control as well as treatments decreased significantly \((p<0.05)\) with progression of storage. Kanagaraju and Subramanian (2012) \([16]\) also reported significant \((p<0.05)\) decrease in overall acceptability scores of spent duck pickle stored at ambient temperature for 90 days.

Control as well as treatments were acceptable till the end of the storage. However, HY had significantly higher overall acceptability scores than control and other treatments. HY also had higher oxidative stability as also observed in present study due to presence of antioxidant and antimicrobial compounds viz. hydrogen peroxide, antioxidants, lysozyme, polyphenols, phenolic acids, flavonoids, methylglyoxal, and bee peptides in honey (Israilli 2014) \([14]\).
Fig 1: Control and hurdle technology based shelf stable chicken pickle on –A- 0 day; B- 30th day; C-60th day

Fig 2: Titrable acidity (Mean ± SE) of hurdle technology based shelf stable chicken meat pickle during storage at ambient temperature

Fig 3: TBARS values (Mean±SE) of hurdle technology based shelf stable chicken meat pickle during storage at ambient temperature
Fig 4: FFA values (Mean±SE) of hurdle technology based shelf stable chicken meat pickle during storage at ambient temperature

Fig 5: Sensory score of hurdle technology based shelf stable chicken meat pickle during storage at ambient temperature
Conclusion
The pH, TBARS, and FFA values of treatments incorporated with humectants were significantly lower whereas titrable acidity was significantly higher than control during storage. The values of titrable acidity, TABRS, and FFA values of control as well as treatments increased significantly whereas pH and scores of all sensory attributes decreased significantly with progression of storage period. However, all the products were well accepted up to 60th day of storage on the basis of sensory score and physico-chemical properties. Among the treatments, HY had higher oxidation stability along with significantly higher overall acceptability scores due to desirable flavour, taste, and texture of product maintained till the end of the storage. Hence, it can be concluded that the storage stability of hurdle technology based chicken pickle might be improved with incorporation of 1% lactic acid as acidulant and 6% honey as humectant. This product may be very well acceptable at room temperature for 60 days on the basis of physico-chemical properties and sensory evaluation done by semi-trained panellists.

Declarations
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Conflict of interest
I certify that there is no actual or potential conflict of interest in relation to this article.

Competing interests
The authors declare that they have no competing interest.

Compliance with ethical standards
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Authors’ contributions: SS conducted all the experiments and wrote manuscript; MG and SS designed the study; SS, Vivekanand and BI edited the manuscript; Vivekanand helped with data tabulation and statistical analysis, VP and MG supervised the work; AKV provided the facilities for conduction of tests done at Goat Products Technology Laboratory, CIRG, Makhdoom.

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