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Macro-mineral profile of assam non-descript sheep (*Ovies aeris*)

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Keywords:

Sheep
Plasma
Macro-Mineral
Reference Value.

Abstract

The present study was carried out to determine the blood macromineralprofile of Assam local sheep grazing in similar pasture. For the investigation, plasma sample was taken for assessing the level of Ca, P, Mg, Na, K, and Cl, which were found to be 10.9 ± 0.18 (mg/dl), 7.6 ± 0.27 (mg/dl), 2.6 ±0.04 (mg/dl), 146 ± 1.5 (mmol/l), 4.5 ±0.16 (mmol/l) and 113 ± 1.9 (mmol/l), respectively. The findings may be of use for physiological characterization of the indigenous sheep breed of Assam. It mays also aid in assessing the health as well as supplementation of mineral mixture for proper maintenance of health and optimization of productive performance of the sheep. However, acomprehensive study is warranted on this aspect to evolve the correlations between blood concentrations of these macroelements and productive performance of this breed.

Introduction

Reference values are of great importance for the correct interpretation of biochemical data (Van Ryssen and Bradfield, 1992). Standard serum chemical parameters provide information that serves as the basis for the diagnosis, treatment, and prognosis of diseases. The major and trace minerals have been recognized as essential for the maintenance of normal metabolic states and productivity in animals (Yokus and Cakir, 2006). In animals, a large number of factors such as species, type or race, sex, age, nutritional and health status affect serum chemistry and mineral levels (Garcia *et al*., 2000; Yokus *et al*., 2006). Hence, there is the need of the database for physiological values of species and breed specific biochemical profile of different farm animals. Further, the knowledge of normal values of biochemical constituents of different animals are of academic as well as of practical importance for clinical and experimental interpretations (Pandey *et al*., 2006). In the present investigation, attempt was made to determine the physiological values of some important macor-minerals in Assam local sheep (*Ovies aeris*).

Materials and Methods

A total of 10 clinicallyhealthy Assam local non-descript sheep reared in the Livestock Farm, College of Veterinary Sciences & Animal Husbandry, Central Agricultural University, Selesih, Aizawl, Mizoram was randomly selected for the study. Approximately ten (10) ml of blood samples were collected aseptically once from each of the experimental animal via jugular vein puncture into sterile vials containing K_3 EDTA (1 mg/ml of blood). Subsequently, the plasma was separated from the blood samples by centrifugation at 3000 rpm for 20 min. All the parameters viz. sodium (Na), potassium (K), chloride (Cl), calcium (Ca), phosphorus (Pi) and magnesium (Mg) were analyzed in the plasma. The plasma electrolytes were estimated by using diagnostic kits from M/s Crest Biosystems, India by following standard protocols viz. Na by colorimetric method (Maruna, 1958 and Trinder, 1951), K by colorimetric method (Sunderman and Sunderman, 1959; Terri and Sesin, 1958), Cl by Thiocyanate method (Schoenfeld and Lewellen, 1964), Ca by OCPC method (Bagainski,1973; Gitelman, 1967), Pi by Molybdate U.V. method

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(Gomori, 1942), and Mg by Calmagite method (Gindler and Heth, 1971) by using a UV-Vis Spectrophotometer (Chemito-Spectroscan 2600).

The data obtained were statistically analyzed using one-way ANOVA as per the method of Snedecor and Cochran (1994).

Results and Discussion

Minerals are an important component of animal diets and are required by organisms to utilize other nutrients in the diet (Szefer and Nriagu 2007). In free grazing animals, minerals derived from natural feedstuffs are often inadequate and require supplementation to satisfy animal requirements (Sowande et al 2008). The amounts of nutrients, including minerals, in the forages greatly vary depending on soil, plant species and management factors (Haenlein 1991). Of the nutritional inadequacies, mineral deficiencies have adverse effect on both animal production and health (Schillhorn van Veen and Loeffler 1990). The concentration of plasma electrolytes viz. plasma Na, K, Cl, Ca, Pi and Mg are presented in Table 1.

The calcium and phosphorus are two important and most abundant minerals and play multiple roles for optimum growth, productivity and maintenance of good health in animals. Due to the abundance of these minerals in the body, it is important to understand their normal level and how to meet requirements to ensure that deficiencies and toxicities are not a concern. A dietary calcium, phosphorus ratio between 1:1 and 2:1 is assumed to be ideal for bone formation and growth. The level ofcalcium and phosphorus in Assam local sheepwere found to be 10.9 ± 0.18 mg/dl and 7.6 ± 0.27 mg/dl, respectively; which was slightly in higher side than goat (Yatoo et al., 2013). The level of magnesiumin sheep was recorded to be 2.6 ±0.04 mg/dl, which was relatively lower than goat and cow. Nonetheless, the mean value of serum calcium in ewes in the present study is comparable to those mentioned by Edmundo et al (1982), Hooda (1992) and Sudhan et al (1996). However, the value is higher than those reported by Tajane et al (1990), and lower than those reported by Kaneko (1997), Karim (2000) and Sharma (2004). The mean value of serum phosphorus is comparable to those mentioned by Edmundo et al (1982), Tajaneet al (1990). However, the value is lower than those reported by Hooda (1992), Sudhanet al (1996), Sharma (2004) and Kaneko (1997). The mean value of plasma magnesium is lower than those mentioned by Edmundo et al (1982), Hidroglou et al (1987), Hooda (1992), Sudhan et al (1996), Kaneko (1997) and Sharma (2004).

The mean values of sodium, potassium and chloride of Assam local sheep was found to be 146 ± 1.5, 4.5±0.16 and 113±1.9, respectively.

The mean value of serum sodium is comparable to those mentioned by Kelly (1984) and Sharma (2004). However, the value is lower than those reported by Tajane (1990) and Hooda (1992). The mean value of serum potassium is lower than those reported by Kelly (1984), Tajane (1990) and Hooda (1992). The mean value of serum chloride is comparable to those reported by Kelly (1984) and Sharma (2004).

Present study thus reports the physiological level of some important minerals in Assam local sheep *(Ovis aeris*). The data generated during the current investigation may be useful as reference values for the scientific community as this is the first study of its kind in this indigenous breed of sheep. Further, it may assist the clinicians to assess health status of the sheep as well as in differential diagnosis of clinical conditions.

Table 1: Macro-minerals in blood of Assam local sheep

Mineral (s)	Concentration
Calcium (mg/dl)	10.9 ± 0.18
Phosphorus (mg/dl)	7.6 ± 0.27
Magnessium (mg/dl)	2.6 ±0.04
Sodium (mmol/l)	146 ± 1.5
Potassium (mmol/l)	4.5 ±0.16
Chloride (mmol/l)	113 ± 1.9

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Effect of level of protein and concentrations of amino acids on egg quality parameters in WL layers

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Keywords:

Egg Production
Haugh Unit Score
Lysine, Protein
Threonine.

Abstract

Quality of egg is important not only for processing market by also for long term storage of shell eggs. Protein/ amino acid levels in diet will influence the quality of egg. Hence two experiments were conducted to assess the quality of an egg at various levels of protein and different concentration of lysine and threonine. 528 and 390 birds at the age of 25 weeks were procured separately and randomly allotted into 11 and 13 treatment groups for experiment -1 and 2 respectively. In experiment 1 the birds were fed with low protein (13.36% CP) and medium protein (15.78% CP) diets each with 6 replicates of 8 birds each at 5 different concentrations of lysine. In experiment-2 two protein groups (13.46% CP/0.65% lysine and 15.56% CP/0.60% lysine) with two lysine levels, each protein was supplemented with 6 concentrations of threonine, fed to 5 replicates each with 6 birds. In both the experiments the control was with 17%CP, 0.70% lysine and 66% threonine. Two eggs/replicate/period a total of 132 eggs/period and 130 eggs/period was collected and brake open for quality assessment in experiment 1 and 2 respectively. Out of all egg quality parameters haugh unit score was increased significantly with increasing amino acid concentration in experiment one. But lower the Haugh unit score at high protein, low lysine and high threonine concentrations were observed in experiment 2. No change in other parameters in both the experiments. Basing on these results it can be concluded that concentration of amino acids especially lysine plays a major role in maintaining the egg quality.

Introduction

Sky rocketing feed ingredient cost in poultry production has been a matter of concern to the nutritionist and farmers. It has been reported that one percent reduction in dietary crude protein through improved amino acid formulations, there is a ten percent reduction in nitrogen losses in poultry waste. Low protein diets with required amino acid composition not only reduces the pollution but also minimize the cost of production [1] without effecting the performance of birds. The primary objective of poultry nutrition is to obtain highest level of performance, reduce nutrient burden on the environment and maximise the profits.

Now the concept has been changed from crude protein to ideal amino acid ratio. Lysine is the base for ideal amino acid profile. It emphasize the need to know the lysine requirement for optimal performance and profits. Lysine is the second limiting amino acid after methionine in corn soy diet [2].

Lysine requirement of hens varied from 650 to 900mg/hen per day [3,4] . Some studies [5,6,7] reported that the optimal Methionine+Cystine/Lysine (Met + Cys/Lys) ratio for laying hens was 0.75. If the protein level of diets changes, the natural lysine content also varies. Threonine is the third limiting amino acid for layers. Threonine participates in

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synthesis of protein and several important metabolic products *viz.* glycine, acetyl Co-A, pyruvate [8] and uric acid [9]. Further, threonine aids in formation of collagen, elastin, and antibodies [10].

Egg quality has become an important aspect of egg marketing. Egg quality comprises a number of factors related to the shell, albumin and yolk, quality may be divided into external and internal appearance [11].

Egg quality is determined by its consumer acceptance with respect to several characteristics including cleanliness, freshness, surface area, mass, volume and coefficient of packaging, egg weight, shell quality, yolk index, albumen index, haugh unit and chemical composition [12] .

All egg quality characteristics are affected by several factors including age and genotype of hen, nutrition, type of rearing system and the time of oviposition [13,14].

Retail outlets are now demanding high standards for conventional internal and external quality characteristics. Quality of protein in diet influences the protein constituents of albumen and yolk of egg. Works carried out so far on protein level, amino acid concentrations in diet focused mainly on egg production and economics. Hence there is a need to quantify the influence of protein and amino acid concentration in diets of layers on egg quality.

Therefore, two experiments (section I and II) were conducted to determine the effect of various dietary concentrations of lysine and threonine at various protein levels on egg quality in WL layers.

Materials and Methods

Birds and Dietary Treatments

In experiment I a total of 528 WL layers (BV-300) aged 25 weeks were randomly distributed into 11 treatment groups each with 6 replicates of 8 birds. They were fed 11 types of diets as illustrated in Table 1 such that treatment diets consisted of 5 different lysine concentrations (0.50, 0.55, 0.60, 0.65, and 0.70%) at 2 protein levels i.e. 13.36 (LCP) and 15.78%(MCP) and control diet with 17% CP and 0.70% lysine. All these diets were iso caloric (2700 Kcal/Kg). A constant ratio was maintained between digestible Mehtionine+ Cysitne, Threonine, Tryptophan, Arginine, Leucine, Iso Leucine and Valine (88, 66, 19, 114, 72 and 80) to lysine.

Where as in experiment –II a total of 390 WL layers aged 25 weeks were randomly allocated into 13 treatment groups of 5 replicates each with 6 hens per replicate. Basing on first experiment two basal diets were prepared with two levels of proteins with two levels of lysine (CP at 13.46 with 0.65% lysine and 15.56 with 0.60% lysine) were tested at various concentrations (60, 63, 66, 69,72 and 75% of lysine as threonine) of threonine in diet. To these basal diets L-threonine at 0, 1.2, 2.4, 3.6, 4.8 and 6.0; 0, 0.9, 1.8, 3.6, 5.4 and 7.2 percent was added to basal diets 1-6 and 7-12 respectively. In this experiment control diet was prepared with 17% CP, 0.70% lysine and 66% of lysine as threonine. All these diets were iso caloric (2700 Kcal/Kg). A constant ratio was maintained between digestible Mehtionine+ Cysitne, Tryptophan, Arginine, Leucine, Iso Leucine and Valine (88, 19, 114, 72 and 80) to lysine.

Fluorescent bulbs were used to provide 16 h of light daily including normal day light. The birds were housed in cages with 4 and 3 hens in each cage (18'' × 15'' × 15'') in expt. I and II respectively. Two adjacent cages were used as treatment replicate. These trials were conducted for a period of 20 weeks. Feed and water were provided ad libitum. The protocol of the current study was approved by the Animal Ethics committee of the institute.

Data Collection

Production parameters were recorded. The number of eggs produced with defective or broken shells and the number of shell less eggs were recorded as egg shell defects (ESD), and expressed in relation to the total number of eggs produced.

Egg Quality

During the last 3 days in each period (28days), 2 eggs per replicate were randomly collected to assess egg quality parameters. A total of 132 eggs/period in experiment I (2x6x11) and 130 eggs/period in experiment –II (2x5x13) were utilised for the measurement of component (yolk, albumen, and shell) percentages, shape, and quality. The major axis of the egg was defined as the longest length of longitudinal diameter and minor axis was defined as the longest length of latitudinal diameter, and these 2 parameters for eggs were obtained using Vernier calipers (accurate to 0.01 mm) (SuCe Measuring Instruments Co., Ltd., China). Eggshell thickness was measured using a micrometer (accurate to 0.001 mm) on at least 5 sites, including 3 equatorial sites, one blunt, and one sharp ends of the shell. The yolk color was measure by using Roche yolk color fan. Haugh units were calculated using the following formula: HU = 100log10 (H -1.7 W0.37 + 7.56), where HU = Haugh unit, H = albumen height (mm), and W=egg weight (g).

Statistical Analysis

Data were statistically analysed by one- way ANOVA using SPSS for windows [15]. The significant differences ($p< 0.05$) seen in between means was determined by Duncans [16] multiple comparison test.

Results

Experiment I

There is no significant effect on, ESD, albumin index, yolk color, shell thickness, shell weight, egg shape index by offering the diets with various levels of protein and different concentration of lysine/ threonine in diet. Significant increase in haugh unit score was observed with increase in concentration of lysine at both the protein levels in experiment I, whereas, no significant variation of Haugh unit in low protein/HL group but significant decrease in high protein/LL group was observed in experiment II.

Significant increase in yolk index value with increase in lysine concentration in high protein group,

Table 1: Nutrient Composition (%) of different dietary treatments fed to WL layers (25-44 weeks) in experiment I

D Lysine (%)	0.50	0.55	0.60	0.65	0.70	0.50	0.55	0.60	0.65	0.70	0.70
Crude protein GROUP	CP 13.36%/LCP I					CP 15.78%/MCP II					Control
ME(kcal/Kg)	2700	2698	2699	2696	2702	2698	2698	2699	2699	2696	2697
CP(%)	13.39	13.34	13.40	13.36	13.38	15.76	15.75	15.80	15.79	15.80	17.06
Total Lysine(%)	0.539	0.537	0.537	0.538	0.539	0.571	0.567	0.571	0.578	0.583	0.705
Total M+C	0.488	0.484	0.483	0.473	0.467	0.570	0.569	0.567	0.562	0.559	0.581
Total Threonine	0.486	0.481	0.500	0.506	0.546	0.562	0.559	0.558	0.556	0.555	0.613
Total Tryptophan	0.132	0.131	0.131	0.203	0.248	0.142	0.141	0.141	0.152	0.158	0.167
Total Arginine	0.804	0.801	0.799	0.787	0.782	0.860	0.854	0.858	0.864	0.866	1.005
Total Isoleucine	0.510	0.504	0.502	0.498	0.494	0.604	0.601	0.600	0.598	0.596	0.668
Total Valine	0.633	0.627	0.625	0.614	0.607	0.737	0.733	0.733	0.728	0.725	0.787
Calcium	4.603	4.603	4.603	4.603	4.451	4.605	4.605	4.605	4.606	4.606	4.428
Available Phosphorus	0.457	0.457	0.456	0.455	0.454	0.451	0.450	0.450	0.451	0.450	0.453
Sodium	0.181	0.181	0.181	0.181	0.181	0.173	0.173	0.173	0.172	0.172	0.172
Chloride	0.186	0.186	0.186	0.185	0.185	0.187	0.187	0.186	0.186	0.186	0.185

Table 2: Nutrient Composition (%) of different dietary treatments fed to WL layers (25-44 weeks) in experiment II

Ingredients	Diet (in Kgs)		
	Basal Diet-I	Basal Diet II	Control
d.Thr	60LCP/HL	60 MCP/LL	66
d.Lysine	0.65	0.60	0.70
	D1-D6	D7-D12	D13 (Control)
Metabolizable Energy (kcal/Kg)	2704	2702	2706
Crude Protien (%)	13.46	15.56	17.05
Total Lysine (%)	0.880	0.820	0.860
Total M+C (%)	0.690	0.630	0.740
Total Thr (%)	0.500	0.460	0.600
Total Tryptophan	0.160	0.170	0.180
Total Arginine	0.940	1.200	1.040
Total Isoleucine	0.560	0.510	0.630
Total Valine	0.650	0.660	0.760
Calcium (%)	4.350	4.350	4.350
Available Phosphorus (%)	0.430	0.430	0.440
Sodium (%)	0.190	0.190	0.180
Chloride (%)	0.170	0.160	0.180

Table 3: Effect of various levels of protein and concentration of lysine on egg quality parameters (Experiment I)

Groups	Lys. (%)	Protein (%)	Egg shell defects (%)	Albumin index	Haugh unit score	Yolk index	Yolk color index DSM 3/1108:5.0)	Shell thickness (mm)	Shell weight (%)	Egg shape index
LCP	0.50	13.36	0.684	0.089	84.13^{c}	0.441^{a}	4.179	0.352	9.883	75.23
	0.55	13.36	0.558	0.089	85.30^{bc}	0.437^{ab}	3.936	0.339	9.577	76.59
	0.60	13.36	0.763	0.089	86.58^{abc}	0.429^{ab}	3.776	0.350	9.788	76.99
	0.65	13.36	0.901	0.089	88.42^{ab}	0.432^{ab}	3.635	0.353	9.813	76.43

	0.70	13.36	0.705	0.090	90.23^{a}	0.402^{cde}	3.601	0.353	9.729	75.94
MCP	0.50	15.78	0.701	0.089	85.98^{bc}	0.410^{cd}	4.078	0.352	9.787	77.07
	0.55	15.78	1.110	0.089	86.74^{ab}	0.387^{e}	3.871	0.348	10.04	76.80
	0.60	15.78	0.834	0.089	86.58^{abc}	0.397^{de}	3.803	0.353	9.896	75.84
	0.65	15.78	0.866	0.089	88.73^{ab}	0.399^{de}	3.636	0.354	9.756	76.97
	0.70	15.78	0.981	0.089	88.14^{ab}	0.418^{bc}	3.501	0.349	9.671	76.80
Control	0.70	17.00	0.983	0.088	86.73^{ab}	0.429^{ab}	3.634	0.344	9.632	75.98
	SEM		0.052	0.145	0.467	0.022		0.933	0.335	0.072
	N		6	6	6	6		6	6	6
	P-value		0.558	0.111	0.020	0.002		0.106	0.177	0.149

Table 4: Effect of various levels of protein/lysine and concentration of threonine on egg quality parameters (Experiment II)

Groups	d. Lysine / CP (%)	d. Thr. (As % lys)	Egg shell defects	Albumin index	Haugh unit score	Yolk index	Yolk color index DSM 3/1108:5.0	Shell thickness(mm)	Shell weight (%)	Egg shape index
LCP/HL	0.65/13.46	60	0.506	0.087	91.44^{a}	0.451	3.237	0.379	10.63	79.27
	0.65/13.46	63	0.556	0.088	92.55^{a}	0.449	2.515	0.353	10.80	77.24
	0.65/13.46	66	0.728	0.091	91.42^{a}	0.450	2.737	0.367	10.65	77.47
	0.65/13.46	69	0.690	0.093	92.41^{a}	0.452	2.205	0.368	10.47	77.58
	0.65/13.46	72	0.932	0.089	90.17^{ab}	0.452	2.732	0.367	10.70	77.18
	0.65/13.46	75	0.768	0.088	90.96^{ab}	0.449	2.593	0.363	10.73	77.12
MCP/LL	0.60/15.56	60	0.664	0.085	90.17^{ab}	0.454	2.762	0.397	10.28	77.27
	0.60/15.56	63	0.792	0.089	91.59^{a}	0.450	2.525	0.400	11.26	76.58
	0.60/15.56	66	0.540	0.086	91.93^{a}	0.450	2.499	0.372	10.02	79.47
	0.60/15.56	69	0.486	0.088	89.84^{b}	0.443	2.384	0.384	10.67	75.62
	0.60/15.56	72	0.474	0.086	89.98^{b}	0.452	2.485	0.380	10.73	77.00
	0.60/15.56	75	0.496	0.086	87.66^{c}	0.441	2.194	0.367	10.70	76.78
Control	0.70/17.05	66	0.462	0.088	88.59^{b}	0.449	2.412	0.356	10.26	76.47
	SEM		0.041	0.022	0.264	0.928		0.542	0.061	0.274
	N		5	5	5	5		5	5	5
	P value		0.439	0.118	0.007	0.141		0.151	0.133	0.345

whereas significant decrease in yolk index value with increase in lysine concentration in low protein group were observed in experiment I. No significant variation in yolk index values were observed due to variation in either protein/lysine or threonine concentration in diet in experiment-II.

Discussion

Egg Shell Defects

There is no significant influence on egg shell quality by alterations in diet of birds. This might be due protein /amino acid concentration had no effect on absorption of calcium and formation of shell.

Albumin Quality

Albumin index values were not influenced either by level of protein or concentrations of lysine/ threonine in both the experiment. Whereas significant increase in Haugh unit score with increase in lysine concentration was observed at both the protein levels in experiment I. Haugh unit is a measure of albumen quality and therefore freshness of the egg [17], while [18] proposed measuring albumen height to determine egg quality. These findings were in collaboration with [19], by altering the protein and lysine in diets of Isa-Brown laying hens. Increase in albumin quality during high ambient temperature was observed by supplementation of ascorbic acid [20] and increasing with vitamin E [21, 22].

In experiment II no significant variation in haughunit score at LCP/HL group, but significant decrease in Haugh unit was observed with increased threonine in diet in MCP/LL group. These are on par with the findings of [23], who observed decrease in albumen quality with increasing dietary protein and amino acid content, similarly [24], also reported decreased Haugh unit score with the dietary addition of neem kernel meal. Albumin quality also influenced by addition of different types or cultivars of grains such as pearl millet [25] or wheat [26] in diet.

Yolk Quality

Significant decrease in yolk index values were observed in low protein groups with increase in concentration of lysine in diet, whereas significant increase in yolk index with increase in concentration

of lysine in MCP group. No variation in yolk index in experiment II with variation in protein, lysine and threonine in diet of birds. Dietary changes had no significant influence on yolk colour in both the experiments.

Yolk quality is determined by the colour, texture, firmness and smell of the yolk [27]. Although yolk colour is a key factor in any consumer survey relating to egg quality [27], consumer preferences for yolk colour are highly subjective and vary widely from country to country. The primary determinant of yolk colour is the xanthophyll (plant pigment) content of the diet consumed. Pale yolks can result from any factor which alters or prevents the absorption of pigments from the diet or the deposition of these pigments in the yolk. Yolk color has a considerable influence on egg marketing.

Shell Quality

Diet had no significant influence on shell thickness, shell weight and shell defects. Increase in egg weight over a production period while decrease in egg shell thickness and strength. Egg shell quality depends on egg size and egg weight. Shell strength and thickness were highly correlated to each other.

Shape Index

There is no significant variation in shape index. This may be due to all the birds are of same strain (same genetic potentiality). Same hatch, management also under uniform condition. This indicates that nutrition had no influence on shape index of the egg. This was coincides with the reports of [28].

Conclusions

Haugh unit score of an egg indicates the albumin quality. Albumin is the rich source of protein. This experiment has proven the dietary protein/amino acid concentration had influence on haughunit score. Basing on this trails it can be concluded that other than good management, best practice with respect to bird husbandry, careful egg collection, handling, processing the diet offered to the birds also will influence the quality of the final product.

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Effect of organic acid supplementation on performance of poultry

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Abstract

In recent times, the poultry industry has paid more attention for addressing the public concerns for food and environmental safety. In an attempt to promote exports of poultry products, antibiotics are being withdrawn from the poultry diets around the world. Organic acids are studied as potential alternatives to antibiotic growth promoters. Their action is related to the pH reduction of the intestinal digesta and affecting the gut ecosystem in numerous ways. Intestinal microbiota can be altered as a result of the remarkable antibacterial activity of organic acids and the growth enhancement of non-pathogenic beneficial microorganisms, due to exclusive competition. Antibacterial activity has been widely reported for many poultry pathogens, such as *Salmonella* spp., *Escherichia coli*, *Clostridium perfringens*, *Campylobacter* sps., both *in vitro* and *in vivo*. Apart from the microbiota, diet supplementation of organic acids has trophic effects on the intestinal mucosa, modifying the morphologic characteristics of intestinal villi and crypts and maintaining epithelial integrity. Furthermore, as found recently, organic acids have anti-inflammatory and immune-stimulating properties. Diet acidification increases gastric proteolysis and the utilization of proteins and amino acids, affects pancreatic secretions and mineral absorption. There are also reports for an effect on appetite and palatability of the feed. All these properties attributed to organic acids have either a direct or indirect effect on the performance and health, even though the results presented for poultry lack consistency. Nonetheless, the benefits of organic acids can have practical application in the control of clinical and subclinical conditions, but more research is needed to study these perspectives.

Introduction

The removal of antibiotic growth promoters (AGPs) from poultry diets in the countries of the European Union in 2006 and similar demands in India, has led the researchers to reconsider the complexity of the gut ecosystem and the need to clarify the continuous interaction among the feed ingredients, the host and the intestinal microbiota, as well as to find alternatives to AGPs (Chowdhury *et al*., 2009; Houshmand *et al*., 2011). Among the alternatives, widely studied are the organic acids. The organic compounds having carboxylic groups including fatty acids with the general structure of R-COOH are termed as acidifiers or organic acids.

The use of organic acids as feed additives has a long history in the food preservation process, preventing food deterioration and extending the shelf life of perishable ingredients (Theron and Lues, 2011). In animal feed industry, they were originally added to serve as antifungals, whereas in poultry, they have also been examined for antibacterial activity against *Salmonella* spp. contaminated feed (Dixon and Hamilton, 1981; Thompson and Hinton, 1997).

However, there are substantial differences in the effect of different organic acid additives. Acetic acid

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and propionic acid show moderate effect on feed pH, while citric acid, formic acid, fumaric acid, lactic acid lower the feed pH substantially. Moreover due to different chemical structures, different acids have different chemical properties. For example, formic acid is active at low concentration against *E.coli* and effectively eliminates salmonellae from the feeds, whereas lactic acid producing bacteria and moulds are relatively resistant to its effect. Propionic acid is more effective in its action against yeasts and moulds compared to other organic acids. Hence research seeks to optimize the additive effects of a combination of buffered organic acids (Hyden, 2000).

The dietary acidification in swine was found to resemble the effect of AGPs in the gastrointestinal tract of farm animals (Senkoylu *et al*., 2007). In poultry production, organic acids have not gained as much attention as in swine production, because there is lack of consistency in the results and great variability in the performance (Dibner and Buttin, 2002).

However, organic acids have made great contribution to the profitability in poultry production affecting the intestinal microbiota, the mucosa and immune system of the host, the protein digestibility, pancreatic secretion, mineral utilization and as a result, the performance (Adil *et al*., 2010). These special properties of the organic acids as well as the practical perspectives of their use are the interesting aspects discussed in this review article.

Antibacterial Activity of Organic Acids

Organic acids enter the gastrointestinal tract in their undissociated form. In this form, they are lipid soluble and able to pass through the cell membrane of the bacterial cell. Once in the cytoplasm of the cell, the organic acids dissociate due to the alkaline environment and release protons (H+) that lower the pH of the cytoplasm. In an attempt to restore the balance, the bacterial cell increases the consumption of adenosine triphosphate (ATP), resulting in a great loss of energy (Paul *et al*., 2007). The anions released (RCOOH-) are responsible for direct antibacterial activities such as damaging the cell membrane, causing leakage and interference in transport of nutrients and disrupting the synthesis of DNA and proteins (Alakomi *et al*., 2000; Davidson, 2001).

However, the antibacterial result of adding an organic acid in the diet depends on many factors.

- *The pKa of the Organic Acid and the pH of the Surrounding Milieu*

Organic acids are weak acids which mean that they can only be partly dissociated. In order to determine the pH value at which each organic acid is half dissociated, the term of pKa was introduced concerning every organic acid. pKa expresses the acidity of weak acids and along with pH, these values determine the amount of organic acid remaining in the undissociated form, capable of entering the bacterial cell. The antibacterial activity increases when pH reduces. Dibner and Buttin (2002) studied the antimicrobial activity of several organic acids at different pH values. At pH 7.3 little antimicrobial activity was observed whereas at pH 4 all acids had better activity against *Escherichia coli*.

- *The Antimicrobial Spectrum of Each Organic Acid*

Studies have shown that propionic acid has better antifungal properties than other acids, whereas lactic acid is more effective against bacteria. Formic acid has been reported to have a broader antibacterial spectrum (Partanen and Mroz, 1999; Haque *et al*., 2009). These differences are the reason why blends of organic acids are most commonly used in poultry feed. However, despite the fact that the organic acids spectrum has been widely studied for bacteria and some pathogenic fungi and yeast like *Aspergillus* spp. and *Candida albicans* respectively (Haque *et al*., 2009; Samanta *et al*., 2010).

- *The Form of the Organic Acids*

When ingested, organic acids disappear in the gastrointestinal tract, being unable to reach parts of the intestine where pathogens inhabit. Hume *et al*., (1993) demonstrated that most of the propionic acid originating from the treated feed is metabolized and absorbed in the foregut of the chicken (crop, gizzard and proventriculus) and does not reach the small intestine or the cecum in sufficient quantities to be effective.

Organic acids have a strong antibacterial effect against *Salmonella* spp. and *E.coli* in the crop which is a major colonization site, but it is desirable to reach further down the intestinal tract in a sufficient concentration. Van Immerseel *et al*. (2004) tried microencapsulation and coating of propionic, formic, acetic and butyric acid in micropearls to allow the slower and selective release of the acidsin the intestine of young chickens.

Effect of Organic Acids on Production Performance in Poultry

Feed Conversion Ratio (FCR) was found to be better

with organic acid supplementation due to decreased feed intake and higher weight gain coupled with improved conditions in the intestines leading to improved digestion, absorption and utilization of nutrients (Parks *et al*., 2001). The reduction of the gastrointestinal pH caused by dietary supplementation of organic acids increases gastric proteolysis, protein and amino acid digestibility. Pancreatic secretions, appetite, palatability of the feed and mineral utilization are also influenced by dietary organic acids (Cave, 1982). These factors along with the properties mentioned above affect zootechnical parameters and performance of poultry.

A positive effect on either feed conversion ratio (FCR) or growth performance has been reported for fumaric, propionic, sorbic and tartaric acid (Vogt *et al*., 1981). FCR was significantly improved by the addition of 1.5% fumaric acid, with lower feed intake compared to the control group. However, body weight gain was not significantly different (Pirgozliev *et al*., 2008). By contrast, Adil *et al*. (2010) found significantly higher weight gain following 3% fumaric acid supplementation, Garcia *et al*. (2007) reported improved FCR with no significant body weight difference feeding 5,000 and 10,000 ppm formic acid, unlike Hernandez *et al*. (2006) and Acikgoz *et al*.(2011) who failed to observe any positive effect on performance of broiler chickens when formic acid was added to the feed or the drinking water respectively. A combination of formic and propionic acid as well as their ammonium salts were found to increase body weight gain and improve FCR. (Senkoylu *et al*., 2007).

Organic acid salts, particularly ammonium formate and calcium propionate, increased live weight and weight gain of broilers until day 21, but no significant differences compared to controls were observed on day 42, although FCR was improved (Paul *et al*., 2007). Esmaeilipour *et al*. (2012) studied the performance of broilers fed 0, 20 or 40 g/kg citric acid for 24days. Addition of 40 g/kg decreased feed intake and body weight gain. This negative effect was also found by Brenes *et al*. (2003), but not by Chowdhury *et al*. (2009) who discerned significant improvement not only on FCR but on body weight as well. Effect of organic acids on production performance of broilers is summarized in Table 1

Table 1: Conflicting results on production performance of broilers with supplementation of organic acids

Effect	Organic acid	References
Improved feed conversion ratio with no difference in weight gain	Fumaric, sorbic, formic, ammonium formate, calcium propionate	Paul *et al*.,2007; Garcia *et al*.,2008; Prigozliev *et al*., 2008
Improved feed conversion ratio and increased weight gain	Butyric, fumaric, lactic, citric, formic, propionic	Leeson *et al*., 2005; Senkoylu *et al*., 2007; Chowdhury *et al*., 2009; Adil *et al*., 2010; Zhang *et al*., 2011
No effect on performance	Formic, fumaric	Hernandez *et al*., 2006; Acikgoz *et al*., 2011
Decreased weight gain	Citric	Bernes *et al*.,2003; Esmaeillipour *et al*.,2012

Vikram Reddy *et al*., (2017) studied the effect of dietary supplementation of organic acids in combination on performance and carcass traits of broiler chicken. Six experimental diets, viz. T1 (Basal diet), T2 (Basal diet+ Antibiotic @ 50 gm/100 kg feed), T3 (Basal diet+20:40:40 combination of citric, formic and propionic acids @ 1.5gm/100 g of feed), T4 (Basal diet+30:40:30 combination of citric, formic and propionic acids @1gm/100g feed), T5 (Basal diet+ 30:30:40 combination of citric, formic and propionic acids @1gm/100 g feed), T6 (Basal diet+ 10:45:45 combination of citric, formic and propionic acids @ 1.5g/100g feed) were prepared. Two hundred and seventy day old, straight run commercial broiler chicks were distributed randomly to six treatments with three replicates of fifteen birds each and fed with the experimental diets from 0 to 42 days of age. Body weight gains and feed efficiency were significantly ($P<0.05$) improved during all phases of the experiment. Feed intake in organic acid supplemented groups (T3 to T6) was significantly ($P<0.05$) reduced during the pre-starter and starter phases. Organic acid supplementation revealed no significant ($P>0.05$) difference in the ready-to-cook-yields among the groups but significantly ($P<0.05$) increased giblet yields on % live weight basis, liver weights, intestinal length and intestinal weight. The organic acid combination of citric, formic and propionic at 20:40:40 combination could be safely incorporated at 1.5% level in broiler diets for better performance.

Effect of Organic Acids on Nutrient Digestibility

Dietary acidification was found to increase the gastric proteolysis, protein and amino acid digestibility and in addition serving as substrates in intermediary metabolism .The improvement in protein digestibility with citric and ascorbic acid was seen to be suggestive of reduction in pH and an

increase in the pepsin activity (Kirchgessener and Roth, 1982).

Reduction in gastric pH follows organic acid feeding which may increase the pepsin activity and the peptides arising from pepsin proteolysis trigger the release of hormones including gastrin and cholecystokinin, which regulate the digestion and absorption of nutrients (Hersey 1987). Furuse and Okumura (1989) found that protein, fat and energy retention were linearly lower as dietary acetic acid was increased from 12.7 to 63.5g/kg diet.

Birds raised on acidified diets exhibited jejunal villi in a zigzag fashion resembling a wave, which facilitated nutrient absorption more efficiently than when they are positioned parallel (Yamauchi and Isshiki, 1991). Abdel-Azeem *et al.,* (2000) concluded that the dietary organic acidification increased the protein utilization and improved its digestibility coefficient. Dibner and Buttin (2002) suggested that organic acids enhanced protein and energy digestibility by reducing microbial competition with the host nutrients, endogenous nitrogen losses, production of ammonia and other growth depressing microbial metabolites. They also minimized the incidence of sub-clinical infections and secretion of immune mediators leading to better performance in the birds.

Thirumeignanam *et al.,* (2006) reported that ileal digestibility of DM, CP, EE and GE were significantly ($P<0.01$) higher in groups supplemented with mixture of organic acid salts at the rate of 1, 1.5 kg/ton in broiler diets compared to unsupplemented and antibiotic supplemented groups. Ghazalah *et al*., (2011) observed that the digestibility of EE, NFE, as well as the ME were significantly ($P<0.05$) improved with organic acids supplementation, while there were no significant differences for digestibility of CP and CF compared to control group.

Ramigani *et al*., 2015 studied the effect of dietary supplementation of organic acids on digestibility of nutrients and serum biochemical profile of broiler chicken. Six experimental diets, T1 (basal diet), T2 (basal diet + furazolidone @ 50g/100 kg feed), T3 (basal diet + 20: 40: 40 combination of citric, formic and propionic acids @ 1.5g/100 g of feed), T4 (basal diet+30: 40: 30 combination of citric, formic and propionic acids @ 1g/100 g feed), T5 (basal diet + 30: 30: 40 combination of citric, formic and propionic acids @1g/100g feed), T6 (basal diet + 10: 45: 45 combination of citric, formic and propionic acids @ 1.5 g/100g feed) were prepared. Digestibility of nutrients and serum biochemical parameters were studied during two phases i.e. at the end of 4^{th} and 6^{th} week. Digestibility of DM, CP and EE increased significantly ($P<0.05$) in organic acid supplemented groups (T3 to T6) compared to T1 and T2 during both stages of the experiment but no significant difference was observed in ME values and CF digestibility. Significantly ($P<0.01$) higher serum total protein, albumin, globulin and calcium levels were recorded in organic acid supplemented groups in both the phases. Serum cholesterol levels decreased ($P<0.01$) in organic acid supplemented groups (T3 to T6) compared to T1 and T2.

Effect of Organic Acids on Poultry Pathogens

Many researchers have studied the effect of organic acids against *Salmonella spp*. in poultry. Formic acid alone or in combination with propionic acid at concentrations of 0.6 % managed to prevent *Salmonella gallinarum* infection (Berchieri and Barrow, 1996). The same combination had a bactericidal effect for *Salmonella enteritidis* when tested *in vitro* with hen's crop contents (Thompson and Hinton, 1997). In an experiment with broiler chickens, Izat *et al*. (1990) found reduced number of *Salmonella* spp. in caecal contents following addition of either 0.36% calcium formate or 0.5% formic acid. Waldroup *et al*. (1995), in contrast, found that formic and propionic acid blend, citric, lactic, fumaric acid in concentrations up to 2% offered no protection for *Salmonella tymphimurium* caecal colonisation. In the last decade, butyric acid was intensively studied for its role in *Salmonella* infections in poultry. Van Immerseel *et al*. (2004) reported the decrease of *S. enteritidis* invasion in caecal epithelial cells *in vitro* after pretreating the cells with butyric acid. On the contrary, pretreatment with acetic acid resulted in increase of invasion.

Invasion of intestinal epithelial cells is an important step in the pathogenesis of *Salmonella*-mediated enteritis and requires a set of genes encoded on the *Salmonella* pathogenicity island1 (SPI1).

Gantois *et al.* (2006) managed to show that butyrate down-regulates SPI1 gene expression, enlightening one of the mechanisms causing reduced invasion. *E. coli* was decreased with the inclusion of propionic acid in broilers feed (Izat *et al*., 1990). Samanta *et al*. (2010) reported a slight reduction of *E.coli* in broilers fed a blend of orthophosphoric, formic, propionic acid and calcium propionate in powder form for 35 days. The most challenging pathological condition, however, seems to be necrotic enteritis, since the ban of AGPs has resulted in outbreaks of the disease and even worse, in lack of ways to control the subclinical cases.

Gauthier *et al*. (2007) evaluated the effect of two microencapsulated blends of organic acids and natural identical flavors in controlling necrotic enteritis in broilers. The first microencapsulated blend

consisted of fumaric, malic, citric and sorbic acid and managed to lower the mortality rate of the infected chickens significantly. The second blend consisted of fumaric acid, calcium formate and calcium propionate and failed to reduce mortality of chickens. The authors assumed that the lower mortality rate in the first group was due to the lower *C. perfringens* numbers in the small intestine and ceca of the broilers. Kocher and Choct (2008) used two mixes of acetic, lactic, fumaric and benzoic acid to test whether the proliferation of *C. perfringens* be controlled, but the results were not that encouraging, especially when compared to those of antibiotics.

In order to demonstrate the effects of organic acids on necrotic enteritis more *in vitro* and *in vivo* studies are needed. Since necrotic enteritis is interdependent with *Eimeria* spp., it would be very useful to know any possible effect of organic acids on coccidia. There have been attempts to study the anticoccidial effect of organic acids, based on performance, mortality rates, lesion scoring and oocyst shedding (Leeson *et al*., 2005; Taherpour *et al*, 2012). The results indicate a complex potential role of organic acids hence, more data both *in vitro* and *in vivo* are necessary to reach to conclusions.

Ramigani *et al*., 2015 studied the effect of dietary supplementation of organic acid combinations on gut pH and *E. coli* count of intestinal contents in broilers. Six experimental diets T1 (Basal diet), T2 (Basal diet+ Antibiotic @ 50 g/100 kg feed), T3 (Basal diet+20: 40: 40 combination of citric, formic and propionic acids @ 1.5 g/100 g of feed), T4 (Basal diet+30: 40: 30 combination of citric, formic and propionic acids @ 1 g/100 g feed), T5 (Basal diet+ 30: 30: 40 combination of citric, formic and propionic acids @ 1 g/100 g feed), T6 (Basal diet+ 10: 45: 45 combination of citric, formic and propionic acids @1.5 g/100 g feed) were prepared. The pH values of crop, duodenum and *E.coli* counts of intestinal contents decreased ($P<0.01$) in organic acid supplemented groups (T_3 to T_6) compared to T_2 and T_1 during both the phases but no significant difference was found in ceacum pH among treatments.

Organic acid combinations supplementation to broiler diets resulted in improvement of acidic environment in the gut of broilers and decrease of microbial load especially during the starter phase. The organic acid combination of citric, formic and propionic at 20: 40: 40 combination can be safely incorporated at 1.5% level in broiler diets for better productive performance.

Effect of Organic Acids on Intestinal Mucosa

Short Chain Fatty Acids (SCFAs) have a proven trophical effect on intestinal mucosa, first described by Frankel *et al*. (1994). Tappenden *et al*. (1994) managed to show that systematic SCFAs can rapidly up regulate the expression of proglycagon and early response genes (c-myc, c-jun and c-foc). Proglycagon-derived peptides are strongly correlated with cellular proliferation in the intestine, while early response genes control cell division, growth, differentiation and apoptosis. Among the three major SCFAs, butyrate seems to have the most stimulating effect on enterocytes proliferation, followed by propionic acid (Scheppach *et al*., 1995). Apart from that, butyric acid is the most preferred source of energy for colonocytes and has been shown to decrease intestinal epithelial permeability by increasing the expression of tight junction proteins (Van Immerseel *et al*., 2010).

Leeson *et al*. (2005) compared the effect of 0.2% butyric acid and bacitracin on crypt depth, finding a significant decrease in duodenal crypt depth of bacitracin treated birds, but no significant difference between the butyrate treated and the control group. That result is in accord with Adil *et al*. (2010), but not with Antogiovanni *et al*. (2007), who observed an increase in crypt depth in the jejunum feeding butyric acid glycerides at the same concentration (0.2%), while the villi were shorter but with longer microvilli (increased density).

Trophic effects of formic acid on the intestinal epithelium are indicated but that requires further research to be confirmed. Unlike SCFAs, the effect of the rest of organic acidifiers is attributed to the inhibition on growth of many pathogenic and nonpathogenic bacteria that prevents inflammation at the intestinal mucosa and damage of epithelial cells. Therefore, nutrient absorption, functions of secretion and energy utilization are improved. However, the form and type of organic acids is believed to influence

Table 2: Effect of Supplementation of Organic acids on Intestinal Mucosa

Effect	Organic acid	References
Trophic effects	Short Chain Fatty Acids	Frankel *et al*.,1994; Tappendan *et al*.,1994
Decreased permeability	Butyric	Van Immerseel *et al*.,2010; Van Deun *et al*., 2011
Increased villus height	Butyric, fumaric, lactic, orthophosphoric, formic, propionic, calcium propionate	Pelicano *et al*., 2005; Senkoylu *et al*., 2007; Adil *et al*.,2010; Samanta *et al*., 2010
Decreased villus height	Butyric glycerides	Antogiovanni *et al*., 2007
Deeper crypts	Formic, butyric glycerides	Antogiovanni *et al*., 2007; Garcia *et al*., 2007
No effect	Butyric, propionic, formic	Leeson *et al*., 2005; Owens *et al*., 2008; Esmaeilipour *et al*.,2012

the effect on gut histology. This may be the reason why supplementation of citric acid in 3 concentrations (0, 20, 40g kg-1) had no effect on intestinal histomorphology (Esmaeilipour *et al*., 2012). Despite the generally accepted fact that organic acids enhance the integrity and effectiveness of intestinal mucosa, more research is needed to examine that effect under both viral and parasitic conditions, harming the intestinal cells. A summary of the organic acids and possible effects on the intestinal mucosa are in Table 2.

Effect of Organic acids on Immune System

The intensive conditions established in the poultry industry demand an active and efficient immune system. There are several studies on the effect of organic acids on immunological responses and immunocompetence of birds. Organic acids have been found to stimulate specific and non-specific gut immune functions (Friedman and Bar-Shira, 2005). Stimulation of humoral immunity has been measured by gamma globulin levels by Rahmani and Speer (2005), who found increased serum gamma globulins adding 2% citric acid in broiler chickens' diet.

These results are in accordance with those of Abdel-Fattah *et al*. (2008), who used acetic, lactic and citric acid in 1.5% and 3.0% concentrations and recorded significantly higher serum globulins. Citric acid though had lower effect compared to acetic and lactic acid, but still higher levels of ã globulins compared to the control group.

Following Katanbaf *et al.* (1989), who reported that increase of spleen, bursa and thymus relative weight is an indicator of immunological advances, acetic, citric and butyric acid were studied on this respect.

Table 3: Effect of Organic acids on Immune System

Effect	Organic acid	References
	Non-specific immunity	
Enhanced mucin production, anti-inflammatory properties, stronger defense barrier	butyric	Van Immerseel *et al*.,2010; Vieira *et al* ., 2012
Enhanced host defense peptide gene expression	butyric	Sunkara *et al*., 2012
	Specific immunity	
Promote humoral immunity	Citric, acetic, lactic, butyric	Rahmani and Speer., 2005; Abdel-Fattah *et al*., 2008
Increased relative weight of bursa and thymus	Citric, acetic, lactic,	Abdel-Fattah *et al*., 2008
Increased density of immunocompetent cells	Citric	Chowdhry *et al*., 2009

Supplementation of all three organic acids was found to increase primary lymphoid organs relative weight (thymus and bursa) compared to the controls, but this effect was not attained for spleen relative weight among all groups (Abdel-Fattah *et al*., 2008). Chowdhury *et al*. (2009) added 0.5% citric acid in a basal diet and found an improvement on immune status, detected by densely populated immunocompetent cells in the lamina propria and submucosa of caecal tonsils and ileum and also in the cortex and medulla of bursa-follicles. A summary of organic acids and possible effects on the immune system are in Table 3.

Conclusion

Summarizing the published data presented in this review article, it can be concluded that organic acids have valuable properties affecting the gut ecosystem and the performance of poultry. If used correctly along with management and bio-security measures, they can even serve as growth promoters, although there is not always agreement on the proper concentrations, the specific age or duration of feeding organic acids and the safety levels.

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Nutritional management of the transition dairy cow for optimal health and production

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Abstract

Feeding and management during the transition period i.e. three weeks immediately before and three weeks after calving, has a significant influence on milk production and fertility. Therefore, time and money spent during transition should be considered as valuable investments for improving farm profitability. Poor feeding and management during the transition period can result in a host of problems around calving such as dystocia, retained placenta, milk fever (hypocalcaemia), grass staggers or hypomagnesaemia, rapid weight loss and ketosis. Most health disorders occur during this time. Feed intake tends to decline as calving approaches, and does not peak until about 10 to 12 weeks after calving. This is the opposite of cow requirements as nutrient demands increase rapidly in the last two months of pregnancy, and cows achieve peak milk yields about six to eight weeks after calving. When cows dip into a negative energy balance, non-esterified fatty acid (NEFA) and beta hydroxy butyrate (BHBA) levels in the blood increase. This is due to large amounts of body fat being utilized as an energy source to support colostrum or milk production., results is ketosis. Nutrition and management programs during this phase directly affect the incidence of post-calving disorders, milk production and reproduction in the subsequent lactation.

Introduction

The transition period in dairy cows is defined as the last three weeks before parturition to three weeks after parturition (Grummer, 1995). It is characterized by tremendous metabolic and endocrine adjustments that the cows must experience from late gestation to early lactation (Drackley et al., 2001). Perhaps the most important physiological change occurring during this period is the decrease in dry matter intake (DMI) around parturition and the sudden increase in nutrients that cows need for milk production (Drackley, 1999). One week before calving, DMI has been shown to decline, with a drop of approximately 30% occurring in the 24 hours before calving (Huzzey et al., 2007). DMI has been shown to drop by 19% on the day after calving, relative to the day of calving when cows are routinely switched to a high energy diet to support lactation needs (Huzzey et al., 2007). As a result, postpartum intakes are usually less than energy requirements (Bell, 1995) leads ton egative energy balance. In response to negative energy balance, cows mobilize stored triglycerides in the adipose tissue in an attempt to meet energy demands for maintenance and milk production. As a result of these remarkable changes, most of the infectious diseases and metabolic disorders occur during this time (Goff and Horst, 1997; Drackley, 1999). Milk fever, ketosis, retained foetal membranes (RFM), metritis and displacement of the abomasum (DA) primarily affect cows within the first two weeks of lactation (Drackley, 1999).

Physical and metabolic stresses of pregnancy, calving and lactation contribute to the decrease in host resistance during the periparturient period (Mallard et al., 1998). During two weeks before and after parturition the T-cells populations exhibit a significant decline, which contribute to the immune-

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suppression in dairy cows at calving (Kimura et al., 1999). This immunosuppression during the periparturient period leads to increased susceptibility to mastitis and other infectious diseases (Mallard et al., 1998).

Metabolic Changes during the Transition Period

In transition cow after calving, an excessive incidence of metabolic and infectious disorders, cyclic feed intakes, and excessive loss of body condition after calving (Drackley, 1998). Poor reproductive success also may be linked back to problems in the transition period (Ferguson, 2001). As calving approaches, concentrations of progesterone in blood decrease and those of estrogen remain high or actually increase (Grummer, 1995). High circulating estrogen is believed to be one major factor that contributes to decreased dry matter intake (DMI) around calving (Grummer, 1993). During the last weeks of pregnancy, nutrient demands by the fetal calf and placenta are at their greatest (Bell, 1995), yet DMI may be decreased by 10 to 30% compared with intake during the early dry period. After calving, the initiation of milk synthesis and rapidly increasing milk production greatly increases demand for glucose for milk lactose synthesis, at a time when feed intake has not reached its maximum. Because much of the dietary carbohydrate is fermented in the rumen, little glucose is absorbed directly from the digestive tract. Consequently, dairy cows rely almost exclusively on gluconeogenesis from propionate in the liver to meet their glucose requirements. Limited feed intake during the early postpartal period means that supply of propionate for glucose synthesis also is limited. Amino acids from the diet or from breakdown of skeletal muscle as well as glycerol from mobilized body fat contribute to glucose synthesis.

Negative energy balance results in a high ratio of growth hormone to insulin in blood of cows, which promotes mobilization of long chain fatty acids from adipose tissue (body fat). Fatty acids released from adipose tissue circulate as non-esterified fatty acids (NEFA), which is a major source of energy to the cow during this period. The concentration of NEFA in blood reflects the degree of adipose tissue mobilization (Pullen et al., 1989), therefore greater the extent of negative energy balance, the more NEFA are released from body fat and the higher the concentration of NEFA in blood. The liver of cows takes up NEFA from the blood that flows through it. As the concentration of NEFA in blood increases around calving or in early lactation, more NEFA are taken up by the liver (Emery et al., 1992). Once taken up by the liver, NEFA can be completely oxidized to carbon dioxide to provide energy for the liver or partially oxidized to produce ketone bodies that are released into the blood and serve as fuels for other tissues, or reconverted to storage fat (triglycerides).

Feed intake and carbohydrate status of the cow are important in determining the extent of body fat mobilization, fatty liver, and ketone body production in the liver. The sudden start of milk synthesis in the udder results in a tremendous demand for calcium. As a result, blood calcium concentrations can drop precipitously at calving, leading to milk fever. Smaller decreases in blood calcium, called sub-clinical hypocalcemia. Hypocalcemia also leads to increased secretion of cortisol, which is believed to be a factor in increased incidence of retained placenta (Goff, 1999).

Metabolic acidosis caused by a negative dietary cation-anion difference (DCAD) favors mobilization of calcium from bone, whereas high dietary potassium concentrations and positive DCAD suppress this process (Horst et al., 1997).

Function of the immune system is depressed during the transition period (Mallard et al., 1998). Decreased ability of the immune system to respond to infectious challenges likely is responsible for the high incidence of environmental mastitis around calving, as well as the high incidence of metritis. Reasons for the decreased immune function are not well understood. Vitamins A and E as well as a number of the trace minerals (selenium, copper, zinc) play a role in enhancing immune function. Recent evidence suggests that negative energy balance may be a major contributing factor (Goff, 1999).

Glucose and Lipid Metabolism During Transition Period

Glucose and amino acids are the major fuel supply of the developing fetus in ruminants. Glucose and amino acids are also needed by the mammary gland for lactose and milk protein synthesis, respectively (Herdt, 2000). Ruminants are not entirely dependent on dietary glucose; as a result they are in a constant stage of gluconeogenesis (Herdt, 2002). The liver serves as a linchpin in adaptation to the maintenance of body fuel supplies and consequently it is the key regulator of glucose supply to the tissues (Herdt, 2000). The major gluconeogenic precursor in ruminants is propionic acid produced in the rumen. Hepatic propionate metabolism is modulated during the transition period. As an example, hepatic blood flow in cows increases 84% from 11 d prepartum to 11 d postpartum (Reynolds et al., 2000). Amino acids, lactate and glycerol are secondary substrates for gluconeogenesis in ruminants (Herdt, 2002).

Excessive lipid mobilization from adipose tissue is linked with greater incidences of peri-parturient health problems like fatty livers may occur in ketotic cows nearly a half-century ago (Saarinen and Shaw, 1950). Fat mobilization syndrome may occurs in early lactation, in which cows mobilized body lipids from adipose tissue and deposited lipids in the liver, muscle, and other tissues. The elevated NEFA concentrations during the last 7 d before calving were associated with greater incidences of ketosis, displaced abomasum and retained fetal membranes but not of milk fever. Metabolism of NEFA by the liver is a critical component of understanding the biology of the transition cow. Extreme rates of lipid mobilization lead to increased uptake of NEFA by liver and increased triglyceride accumulation (Figure 1). If this lipid infiltration becomes severe, the syndrome of hepatic lipidosis or fatty liver may result, which can lead to prolonged recovery for other disorders, increased incidence of health problems, and development of "downer cows" that may die. Increased lipid accumulation and decreased glycogen in the liver were associated with an increased susceptibility to induction of ketosis.

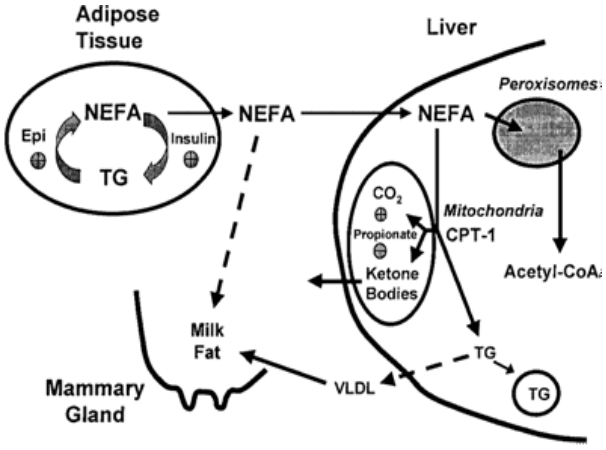

Fig. 1: Schematic representation of relationships among lipid metabolism in adipose tissue, liver, and mammary gland

Animal Management during Transition Period

Nutritional Management

To maximize productivity and ensure successful reproduction, rations fed during this time need to be nutrient dense and often contain more expensive ingredients. Therefore, a poor nutritional program during the transition period increases feed costs per unit of milk produced and decreases income through lost milk production, decreased reproductive efficiency, and increased incidences of metabolic disorders. Interest in nutrition and management of dairy cows during the transition period has increased dramatically during the last decade as researchers and field nutritionists have recognized the importance of this critical six week period (Drackley, 1999).

Nutritional transition management consisting of three distinct phases for the cow: Phase 1 is the far-off dry period. This phase has relatively simple nutritional requirements but should not be ignored. Phase 2 is the close-up period, when many of the metabolic changes for lactation actually occur. Phase 3 is the fresh cow, which completes the transition into full lactation. The new National Research Council (NRC) publication Nutrient Requirements of Dairy Cattle (NRC, 2001) has incorporated much new information regarding the transition period, and has made recommendations for nutritional management of transition cows during these three phases. The general concept of ration changes during the transition is that nutrient density is increased gradually from that fed to far-off dry cows to the higher nutrient density required for fresh cows. Because DMI of close-up cows declines by 10 to 30% during the last 7 to 14 days before calving, nutrient density must be increased to compensate.

The balance between structural carbohydrates (fiber) and nonstructural carbohydrates (grains or concentrate by-product feeds) in diets fed before and after calving is probably the most important dietary factor for transition success. Adequate fiber of sufficient particle size is needed to maintain rumen function, prevent acidosis and displaced abomasum, and achieve high DMI. On the other hand, excessive neutral detergent fiber (NDF) content may limit intake. Sufficient non-fiber carbohydrates must be present to provide adequate energy in the form of propionic acid for glucose synthesis and to suppress synthesis of ketone bodies. Another benefit of additional grains in the pre-partum diet is to adapt the ruminal tissues and the rumen microbial population to the type of diet that will be fed after calving (Goff and Horst, 1997). Grain feeding increases length of the rumen papillae in comparison to feeding only poorly digestible roughages (Dirksen et al., 1985). To prevent periparturient diseases five areas are for concern maximizing dry matter intake, stimulating rumen papillae development, minimizing negative energy and protein balance, maintaining protein homeostasis, minimizing immune dysfunction. It is in the best interest of dairy farmers to reduce disease during the transition period for both their economic survival and for the welfare of their cows. A key challenge for veterinarians is to educate dairy producers to devote adequate resources in terms of labor, facilities and management to implement a structured transition cow program. One approach is

to build an economic basis for this approach. It is generally accepted that a good dry cow program will result in an additional 1,000 to 2,000 lbs of milk in the next lactation. At least a portion of this production response is due to a decrease in post-calving disorders.

To prevent periparturient diseases and increase the potential for successful reproduction, there are five critical control points during transition period that need to be addressed.

- *Maximizing Dry Matter Intake*

Cows that experienced periparturient disease have shown that there was a greater decline in dry matter intake. Restricting DMI in the dry period allows cows to increase DMI immediately postpartum, resulting in higher energy balances, and decreased body fat mobilization, evident by lower NEFA (non-esterified fatty acid) and BHBA (beta hydroxyl butyrate) concentrations (Dann et al., 2006).

The National Research Council (2001) reported the following prediction equations for DMI during the last 21 days of gestation:

Heifers: DMI (% of BW) = $1.71 - 0.69\ e^{0.35t}$

Cows: DMI (% of BW) = $1.97 - 0.75\ e^{0.16t}$

Where: "t" = days of pregnancy minus 280.

Increasing dietary NFC (non-fibre carbohydrates) or decreasing NDF (neutral detergent fibre) during the transition period stimulates DMI. When energy density of the diet increased from 1.3 to 1.54 Mcal ENI/kg DM and crude protein increased from 13 to 16% at about 3 weeks prior to calving, DMI increased in 30% (Emery, 1993).

- *Stimulating Rumen Papillae Development*

Rumen papillae helps to maintain acid-base balance in the rumen by absorbing volatile fatty acids and especially lactate, generated by microbial fermentation. Growth of these papillae is influenced by the presence of fermentation products, primarily propionate and butyrate and not acetate. Higher fibre diets predominately produce acetate during fermentation, which results in a reduction in papillae length. Adding fermentable non-structural carbohydrates to the late gestation diet can have positive effects by initiating rumen papillae growth and allowing rumen organisms to adapt to the starch substrate.

- *Minimizing Negative Energy and Protein Balance*

Excessive energy intake leads to 'fat cow syndrome'. Feeding gluconeogenic precursors such as propylene glycol has also shown positive effects on energy status of the late pregnant cow. Prepartum protein depletion adversely affects periparturient metabolic status, resulting in a greater incidence of ketosis and fat cow syndrome. Energy balance of a transition cow is determined by subtracting energy requirements for maintenance and gestation from energy intake. During the transition period, feed intake is decreasing at a time when energy requirements are increasing due to growth of the conceptus. Increasing the energy and protein density up to 1.6 Mcal of NEI/kg and 16% CP in diets during the last month before parturition improves nutrient balance of cattle prepartum and decreases hepatic lipid content at parturition.

- *Maintaining Calcium Homeostasis*

The onset of lactation causes a severe and rapid drain on blood calcium required to produce milk. If this blood calcium is not replaced as rapidly as it is reduced via bone calcium release (resorption) or intestinal absorption of calcium, cows will become hypocalcaemia with some developing clinical milk fever. Reducing DCAD (dietary cation-anion difference) to negative values has been shown by many authors to prevent this rapid decline in blood calcium at calving.

- *Minimizing Immune Dysfunction*

The immune system of the cow has been shown to decline in response to the transition period, possibly as a result of increased cortisol secretion associated with stress of late gestation and calving. Neutrophils are a type of white blood cell involved in the first line of defence against infection (Frandson et al., 2006). It has been reported that the function of neutrophils is impaired in transition dairy cows leading to a state of immune-suppression. Elevated blood NEFA concentrations before calving have been linked with uterine disorders and impaired neutrophil function (Hammon et al., 2006). Micro minerals and vitamins supplementation recover from immune function problem.

Conclusions

The transition period is a critical determinant of both productivity and profitability in a dairy herd. Nutrition and management programs during this phase directly affect the incidence of post-calving disorders, milk production and reproduction in the

subsequent lactation. The transition period imposes a number of abrupt changes on the cow. The cessation and initiation of lactation is one example. Rapid changes in both hormonal and metabolic systems must occur. All of these tend to increase the level of stress in the cow during this period. The stress response mechanism in ruminants is a complex, multifaceted system. Nutrition and management alterations provide an opportunity to minimize the effects of stress. Attention to keeping cows as comfortable as possible during the transition likely is as important as the nutritional management program.

Future Prospects

To obtain maximum profits, nutritionists and veterinarians should work together with dairy producers to design practical strategies to help cows make smooth transitions, so that cows produce to their potential during early lactation.

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Viable mutants in blackgram

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Abstract

Pulses are an important role in human dietary. Pulse protein provides good supplements to cereal diets and enhances the biological value of protein when consumed. Pulses are often attributed as "Poor man's diet". Crop improvement programme that the natural variability is an essential pre-requisite, however in blackgram due to their autogamous nature it lack genetic variability. Hence the present mutation breeding programme was taken up gammy ray irradiation to identify mutants with high yield potential, early maturity, and disease resistance and bold seeded type.

Pulses is an important role in human dietary. Pulse protein provides good supplements to cereal diets and enhances the biological value of protein when consumed. Pulses are often attributed as "Poor man's diet". Among the pulse blackgram (Vigna *mungo*) is an important kharif pulse in India grown on about 2.4 lack heatures. The annual production of blackgram is 10.32 lakh tonnes from an area of 27.56 lakh hectares in Tamilnadu and the productivity is 480 Kg ha^{-1}.

Any breeding programme that the natural variability is an essential pre-requisite, but in blackgram due to their autogamous nature it lack genetic variability. Mutation breeding is suitable choice of creating variability in self pollinated crops like blackgram. Therefore, the present mutation breeding programme was taken up to identify mutants with high yield potential, early maturity, and disease resistance and bold seeded type.

Seeds of ADT 3, ADT 5 and APK 1 varieties were subjected to 10 to 100 KR with an interval of 10 KR of gamma ray irradiation at IGARC, Kalpakkam during July, 2001. For each dose of physical mutagen a random sample of 370 seeds were treated in each variety. A total of 270 seeds in each treatment were sown in the field under Randomized black design in three replications with a spacing of 30 cm between rows and 15 cm between plants.

The individual M_1 plants were harvested separately and the seeds were sown as plant to row progenies to raise M_2 generation. Visual observations were made to isolate different morphological mutants.

The viable mutants were scored in M_2 generation based on their phenotypic changes or expression and changes in qualitative characters. They are categorized into several groups as stature, duration, leaf, pod seeds and sterile mutants. The characteristics of the mutants isolated in the present study are as follows.

Tall Mutant

Tall mutants were observed at 60, 80 KR in ADT 3, 50 and 70 KR in ADT 5 and 40, 70 KR in APK 1. Tall plants did not show any variation in the number of internodes, but they possessed longer internodes and they were erect habit. Similar type of m mutant has been reported by Sinha *et al*. (1969) in mungbean.

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Dwarf Mutant

These mutants noticed were very short height with 12 to 15cm in height at the time of maturity in ADT 3. The height of dwarf mutants in ADT 5, APK 1 is 13 cm and 12-13.5 cm respectively. In ADT 3 dwarf mutant were stunted in growth and gave poor yield. It was reported by Ignacimuthu (1988) in M_1 and M_2 generations of *Vigna mungo*, Mahna *et al*. (1990 a), Gautam and Mittal (1998) in blackgram, Singh and Yadav (1991) in greengram.

Spreading Type

Spreading type mutants were different from the normal type with creeping habit and longer internodes. These mutants have more in the number as well as length of the branches resulting in more spread than the control.

Open and Compact Type

The open type mutants are branched at an angle of 80^0. They had a less number of primary branches. The open type was recorded in all the three varieties. The compact type mutants are bushy habit with more number of leaves, and had condensed internodes.

Early and Late Mutants

Early mutants matured 10 to 15 days earlier than the respective control. Late mutants had the delayed flowering and matured late by 12 to 18 days when compared to control. The lateness may be due to the mitotic arrest in the flower primordia. These duration mutants have been reported by Charumathi *et al*. (1992), Vanniarajan *et al.* (1993b) and Prema Manapure and Santhi Patil (1997) in blackgram.

Table 1: Frequency of viable mutants isolated in M_2 generation:

Mutants	Frequency (%)		
	ADT 3	ADT 5	APK 1
Tall mutant	5.00	3.44	3.03
Dwarf	5.00	5.17	6.06
Spreading	3.33	5.17	4.54
Early mutant	3.33	5.17	3.03
Late mutant	3.33	3.44	4.54
Open	3.33	5.17	4.54
Compact	5.00	3.44	6.06

The useful mutants like tall mutants and early flowering mutants are suggested to be utilized in future hybridization programme.

Conclusion

In M_2 generation a total of 184 viable mutants were observed in all the three varieties. Among these APK 1 had more number of mutants and the variety ADT 5 had less number of mutants. But with respect to doses 80kR had less number of mutants.

Among the viable mutants, more number of stature mutants were recorded in APK1. While comparing different doses of gamma ray more numbers of stature mutants were found at 70kR. Regarding the leaf mutants high proportion was observed at 50 kR level and the all varieties had equal proportion. The seed mutants were high in 60 kR level.

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