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NUTRITIONAL AND PHYSIOLOGICAL STUDIES ON FRIESIAN CALVES FED PROTECTED FAT AND PROTEIN DIETS:
6. HISTOLOGICAL AND HISTOMETRIC CHARACTERISTICS OF THE RUMEN

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ABSTRACT

A total of 18 Friesian calves having one month of age was used in 3 dietary treatment groups to study the effect of feeding protected protein or protected fat diets, from 1 up to 8 months of age, on histological characteristics of the rumen of Friesian calves. The 1st group (control) was fed untreated concentrate feed mixture (CFM), the 2nd was fed CFM, 5% of it was replaced by protected fat (PF) and the 3rd was fed CFM treated with 1% formaldehyde on the basis of its total CP (PP). All groups were fed plus berseem hay beside the CFM. Thickness of all ruminal wall tunicae, mucosal laminae and stratum (st.) corneum was estimated. Also, density and dimensions of ruminal papillae were measured to calculate papillary surface area. Results indicated that tunica mucosa was thicker ($P<0.05$) in PP than in PF and control groups. Thickness of lamina epithelialis mucosa (LEM) did not differ significantly among treatment groups. However, it was thicker ($P<0.05$) in PF than in PP group (0.30 vs. 0.27 mm). As overall mean, st. corneum tended to be thicker in control (44.3 µm) than in PF (42.9 µm) and PP (43.2 µm) group. Lamina propria was thicker ($P<0.05$) in PP than in PF and control groups (1.20 vs. 0.96 and 1.02 mm). Submucosa was thicker ($P<0.05$) in PF and PP groups than in control group. Total thickness of tunica musculosa was higher ($P<0.05$) in PF and PP than in control group. Generally, thickness of all previous traits varied significantly between different ruminal sacs as affected significantly by dietary treatments. Ventral ruminal sac showed significantly the highest thickness in calves of all groups. Papillary length was affected ($P<0.05$) by dietary treatment, but each of PF or PP group did not differ significantly from that of the control one. Papillary length in was shorter ($P<0.05$) in PF than in PP group. Treatment group did not affect papillary width. Density of papillae was lower ($P<0.05$) in PF and PP groups than in control one. Surface area of each papilla was lower ($P<0.05$) in PF than in PP and control groups, which did not differ significantly. Papillary surface area per cm^2 was lower ($P<0.05$) in PF group; however, PP group showed insignificantly lower values compared with the control. Inter-papillary surface area per cm^2 showed negative relationship with papillary density and was significantly affected by dietary treatments, ruminal sac and their interaction. Total surface area per cm^2 of different ruminal sacs mainly affected by surface area of papillae within each cm^2. So, total surface area/cm^2 showed the same trend of surface area of papillae/cm^2 as affected by dietary treatment and ruminal sacs. On the basis on the foregoing results, it could be conclude from the nutritional point of view that feeding calves on the tested diets resulted had beneficial effects on histological and histometric characteristics of rumen of Friesian calves during suckling period and early post-weaning ages.

Keywords: Histological, rumen, calves, protected protein, protected fat,

INTRODUCTION

Nutritional status of suckling calves during the first period of their life is the major managerial factor affecting their future productivity. Therefore, the growth performance of individual calves at earlier ages, particularly during suckling period, is crucial factor in determining the rumen capacity of calves at the later ages. Incorporation of protected protein in diets is recommended in highly producing animals (NRC, 1984). Dietary protected protein increased growth rate and nitrogen retention in ruminants (Itabashi *et al.*, 1994 and Virk *et al.*, 1994). Feeding protected protein results in pronounced alteration in rumen fermentation (El-Reweny, 1999), which may lead to affect the morpho-histological parameters of the rumen (Abdel-Khalek, 2000). Using protected fat in ruminants feeding is of interest for several reasons; among these are: its high energy density (Omer, 1999), its buffering capacity of ruminal condition (Ohajuruka *et al.*, 1991) reducing feeding costs and reducing environmental pollution (Omer, 1999). There are some advantages of using Ca-salts of fatty acids in ruminants feeding including protection of dietary fat against rumen degradation as it is an insoluble soap, and it is a compound of fatty acids which are more digestible than the fat itself. However, the information on the effect of dietary protected fat on histological parameters of the rumen are rare.

This study was undertaken to investigate the effect of feeding protected protein and fat diets on histological and histometric characteristics of rumen of Friesian calves at 8 months of age.

MATERIALS AND METHODS

The present study was carried out at Sakha Animal Production Research Station, belonging to the Animal Production Research Institute, Agricultural Research Center, Ministry of Agriculture, in cooperation with Animal Production Department, Faculty of Agriculture, Mansoura University.

Animals:

Eighteen suckling Friesian calves were used in this study having average LBW of 47.76±1.24 kg and one month of age. Animals were divided into three similar groups, six calves in each according to body weight. Calves in the 1st group were fed on untreated concentrate feed mixture (CFM) and was considered as a control group (G1). Calves of in the 2nd group (G2 were fed on formaldehyde-treated CFM as a protected protein diet (PP), while those of the 3rd group (G3) were fed untreated CFM supplemented with protected fat (PF). Calves were free of any diseases with healthy appearance and they were housed in groups and were kept under semi-open sheds.

Feeding system and management:

The concentrate feed mixture (CFM) used in this study was composed of 37.5% yellow corn, 20% soybean meal, 15 % corn gluten, 22.5% wheat bran, 3% molasses, 0.5% premix and 1.5% common salt. For calves fed PP diet in G2, CFM was treated with 38-40% formaldehyde at a level of 1% on the basis of crude protein level of CFM. However, 5% of CFM

in diet of calves in PF group was replaced by Magnapac (Ca-soap of fatty acids). The Magnapac (NOREISA, Madrid, Spain) contained 84% palm oil (44% palmitic, 40% oleic, 9.5% linoleic, 5% stearic and 1.5% myrstic acid), 12.5% Ca-carbonate and 3.5% moisture.

Calves were allowed to suckle colostrum from their dams throughout the first three days postpartum, thereafter they were artificially suckled twice daily by drinking milk from bucket at 7.0 a.m. and 6.0 p.m. Throughout the experimental period of 7 months, calves in all groups were fed on similar amounts of CFM, milk and berseem hay (*Trifolium alexandrinum*) according to the recommendation of the NRC (1984). Chemical composition of CFM, milk and berseem hay used in feeding calves in all groups is shown in Table (1).

Table (1): Chemical analysis of feed stuffs (% on dry matter basis).

Item (%)	Feed stuff				
	CFM			Milk	Berseem hay
	CFM	TCFM	CFM+PF		
Dry matter, DM	91.58	90.99	91.64	12.80	88.24
Organic matter, OM	90.68	90.71	89.64	94.46	88.19
Crude protein, CP	18.43	18.41	18.37	24.71	14.32
Crude fiber, CF	10.96	10.89	10.62	00	24.67
Ether extract, EE	4.91	5.01	8.52	30.50	6.04
Nitrogen free extract	56.38	56.40	52.13	39.25	43.16
Ash	9.32	9.29	10.36	5.54	11.81

One kg of premix contained 3.3 x 10^6 IU vit. A; 3.3 g vit. E; 3.3 x 10^5 IU vit. D_3; 0.33 g vit. K; 0.33 g vit. B_1; 1.33 vit. B_2; 6.67 vit B_5; 0.50 g vit B_6; 3.3 g vit. B_{12}; 3.3 pantothenic acid; 0.33 folic acid; 16.67 mg Biotin; 166.67 g Colin; 1 g Copper; 10 g Iron; 13.3 g Mn; 15 g Zn; 0.1 g Iodine; 0.03 g Se and carrier $CACO_3$ to 1 kg. TCFM: formaldehyde-treated CFM.

The determined amount of CFM for treatment was mechanically ground and sprayed by an agriculture sprayer with the recommended amount of formaldehyde (1850 ml formaldehyde/ ton CFM) according to the method described by Ferguson *et al.* (1967). During spraying the CFM was manually mingled and was stored in well tight plastic containers and left for the complete reaction of formaldehyde with crude protein in CFM for two weeks at room temperature before using in animal feeding. However, in PF diet, CFM was well mixed with protected fat supplements and stored for feeding calves in protected fat diet group. Chemical analysis of representative monthly samples of the experimental diets was performed according to the official methods of the AOAC (1980).

Slaughter and sampling procedures:

At the end of the experimental period at about 8 months of age, three random calves in each group were slaughtered after fasting for 16 hours. After slaughter, the digestive tract was immediately removed by severing the esophagus that was tied at the esophageal cardiac orifice, and then rumen was separated from the other segments of the gastrointestinal tract according to Abdel-Khalek (1986).

Histological traits:

Rumen was emptied from its content, then cleaned gently by tap water and tissue samples were taken immediately from different parts of the

rumen (ventral, dorsal, cranial, ventral caudal blind, and dorsal caudal blind sacs) for the histological examination. The ruminal tissue samples were immediately fixed, in 10% formalin solution, washed by running tap water and dehydrated in ascending grades of alcohol, cleared in toloul, impregnated in paraffin wax and embedded in paraffin blocks. The paraffin blocks were cut into thin sections (6-8 µ) by a microtome and stained by Haematoxylin and eosin using the routine method after Bancroft and Stevens (1982).

The slides were examined by means of light microscope and all aspects of tunica mucosa and musculosa were taken. A standard micrometer eyepiece was used for measuring thickness of different ruminal wall tunica, (mucosa, submucosa and musculosa). Thickness of different mucosal laminae (epithelialis and propria), stratum corneum and both inner circular and outer longitudinal muscle layers of musculosa as well as length, width, and density of ruminal papillae (Tamate *et al.*, 1962) were estimated. Papillae surface area (PSA) was calculated according to Nocek *et al.* (1984) as the following:

$$PSA/cm^2 = length \times 2\ width \times density\ of\ papillae/cm^2$$

Average cross-section area (ACSA) of the papillae was calculated by measuring mean diameter (smallest and largest) of cross-section area of each papilla as a circular shape, and then total area occupied by papillae per cm^2 was calculated after (Abdel-Khalek, 1986) as the following formula:

$$Total\ area\ of\ papillae/cm^2 = ACSA \times density/cm^2$$

The inter-papillary area was obtained by subtracting the product from 1.0 cm^2 area. The total absorptive surface area per cm^2 of mucosa was calculated as a total papillary surface area plus inter-papillary surface area.

Statistical analysis:

Statistical analysis for the obtained data was performed by the method of analysis of variance according to Snedecor and Cochran (1980) using the general linear model procedures of SAS (1987). Duncan multiple range test (Duncan, 1955) was used to determine the significant differences among age or group means.

RESULTS AND DISCUSSION

Histological characteristics of the rumen:

Tunica mucosa:

In calves of all groups, the ruminal wall was composed of lamina epithelialis and lamina propria, but lamina muscularis mucosa was absent. Tunica mucosa formed branched like-finger papillae with different lengths (Figs. 1, 2 and 3). The ruminal papillae and the inter-papillary space had almost similar structure of tunica mucosa. All ruminal sacs showed the same structure of tunica mucosa, but different in appearance of their papillae. Similar findings were observed by Hemmoda (1981) and Youssef (1992) in Egyptian buffalo calves. The effect of dietary treatment on tunica mucosa of all ruminal sacs was significant ($P<0.05$), being thicker in rumen of calves fed PP diet than those fed PF and control diets (Table 2). In different ruminal sacs, it was found that only feeding calves on PP diet decreased mucosa thickness in the dorsal sac and increased it in the dorsal caudal blind one,

while both ventral and cranial sacs were not affected. Also, thickness of mucosa in all ruminal sacs of calves fed PF diet did not differ significantly from that of the control calves (Table 2).

The differences in thickness of mucosa layer in different sacs were significant ($P<0.05$), being the highest in ventral sac, followed by dorsal and ventral caudal blind sacs. However, cranial and dorsal sacs showed significantly ($P<0.05$) the lowest thickness of mucosa (Table 2). The present differences may indicate higher rate of absorption within the ventral sacs than in the other ruminal sacs.

Lamina epithelialis mucosa (LEM).

In calves of all groups, lamina epithelialis mucosa (LEM) consisted of squamous or cubical stratified type, but degree of keratinization on its surface was different as affected by dietary treatment, being more keratinized in rumen of the control (Fig. 4) than in PF group (Fig. 5). However, LEM in rumen of calves fed PP diet showed slight degree of keratinization (Fig. 6). It is of interest to note that the thickness of LEM in rumen of calves in different treatment groups was associated with color of the interior surface of the rumen, being dark with high thickness in calves fed PF, light with low thickness in those fed PP diet. Wardrop (1961 a and b) described similar findings in rumen of calves. Also, similar observation was found on Egyptian cows and buffaloes as affected by dietary treatment (Hemmoda, 1981). In this respect, Piatkowski (1975) observed that the differences in color of the superficial layer of the ruminal mucosa are independent of the diet.

Thickness of LEM differed significantly ($P<0.05$) in various ruminal sacs, but did not differ significantly as affected by dietary treatment (Table 1). Thickness of LEM varied considerably in different ruminal sacs, showing significantly ($P<0.05$) the highest thickness in dorsal caudal blind and ventral caudal blind sacs, moderate in ventral and cranial sacs, and the thinnest in the dorsal sac. In spite of the insignificant differences among treatment groups, TLEM was the thickest in ventral blind sac and the thinnest in ventral sac of the control calves. This trend changed by feeding calves on PF and PP, being the thickest in dorsal caudal blind sac and the thinnest in dorsal sac of calves fed PF. However in calves fed PP diet, the corresponding trend was found in dorsal blind sac and dorsal sac (Table 2).

These findings indicated pronounced variation in thickness of LEM in different ruminal sacs of calves fed PF or PP diet as compared to those fed the control diet. Khalil (1974) found marked changes in thickness of LEM of lambs fed different concentrate to roughage ratios. Also, Youssef (1992) reported wide variation in thickness of LEM in different ruminal locations of Egyptian buffaloes. Such variation was associated with different absorptive rates of ruminal fermentation products within various locations of the ruminal surface (Abdel-Khalek, 2000).

In general, the changes in the thickness of the lamina epithelialis mucosa have mainly occurred via changes in the number of cell layers (depth) rather than cell dimensions (Abdel-Khalek, 1986). The lamina epithelialis mucosa consisted of four strata, basal, spinosum, granulosum and corneum. The differences in thickness of lamina epithelialis at the earlier ages may be attributed to variation in thickness of st. corneum as affected by

dietary treatment during the suckling period in different species of ruminants (Khali, 1974; Hemmoda, 1981 and Abdel-Khalek, 1986 and 2000). In this respect, Abdel-Khalek (2000) found significant ($P<0.05$) reduction in thickness of lamina epithelialis mucosa in rumen of lambs fed PP diets. Such trend may support the present insignificant decrease in thickness of lamina epithelialis in calves fed the PP diet.

Table (2): Means and standard errors of thickness of mucosa, lamina epithelialis and stratum corneum in different ruminal sacs of calves as affected by dietary treatments.

Group	Ruminal sac					
	Dorsal	Ventral	Cranial	Dorsal caudal blind	Ventral caudal blind	Overall Mean
Thickness of mucosa (mm):						
Control	1.01±0.12	1.35±0.14	1.14±0.10	1.22±0.13	1.20±0.15	1.18±0.03^{b}
PF	1.08±0.08	1.38±0.11	1.03±0.09	0.97±0.07	1.16±0.12	1.13±0.03^{b}
PP	0.77±0.05	1.64±0.28	1.61±0.10	1.61±0.26	1.38±0.17	1.25±0.06^{A}
Overall mean	0.95±0.03^{c}	1.46±0.05^{a}	1.01±0.03^{c}	1.27±0.06^{b}	1.25±0.04^{b}	
Thickness of lamina epithelialis (mm):						
Control	0.28±0.05	0.21±0.01	0.31±0.01	0.27±0.01	0.37±0.02	0.29±0.01
PF	0.21±0.01	0.33±0.01	0.30±0.01	0.35±0.02	0.32±0.01	0.30±0.01
PP	0.17±0.00	0.27±0.02	0.20±0.01	0.41±0.02	0.30±0.01	0.27±0.01
Overall mean	0.23±0.01^{c}	0.27±0.01^{b}	0.27±0.01^{b}	0.33±0.02^{a}	0.33±0.01^{a}	
Thickness of stratum corneum (µm):						
Control	39.1±1.45	40.0±1.50	50.3±1.54	38.9±1.1	53.3±2.2	44.3±1.0
PF	38.9±1.14	40.7±1.21	47.3±1.30	48.0±2.4	39.4±1.2	42.9±0.8
PP	35.8±0.63	38.9±1.12	41.2±0.21	60.1±3.1	40.0±1.2	43.2±1.2
Overall mean	37.8±0.64^{b}	39.9±0.72^{b}	46.3±0.94^{a}	49.0±1.8^{a}	44.3±1.3^{a}	
Thickness of lamina propria (mm):						
Control	0.85±0.05	1.23±0.06	0.97±0.05	1.06±0.06	1.00±0.07	1.02±0.03^{B}
PF	0.96±0.03	1.20±0.05	0.86±0.04	0.77±0.03	0.99±0.05	0.96±0.02^{B}
PP	0.97±0.02	1.48±0.12	0.94±0.05	1.38±0.11	1.21±0.08	1.20±0.05^{A}
Overall mean	0.93±0.02^{c}	1.30 ±0.04^{a}	0.92±0.02^{c}	1.07±0.05^{b}	1.06±0.00^{b}	

A & B and a, b & c: Means having different superscripts within the same row and column, respectively, are significantly different at $P<0.05$.

Stratum corneum:

Stratum corneum of lamina epithelialis mucosa was composed of flattened cells with central small spherical nuclei in all calves. Type of st. corneum cells was in form of either serrated in calves of control (Fig. 4) and PP (Fig. 5) groups or smooth surface in calves of PF group (Fig. 6). The serrated surface may result from the rupturing of ballooned cells of st. corneum, which have a cornified wall and an unstained cytoplasm. Also, st. corneum consisted of elongated and flattened cells in particular at the tops of papillae. The degree of keratinization was more advanced in the control calves (Fig. 4) than in those fed PF and PP diets (Figs. 5 and 6).

In spite the differences in histological feature of st. corneum among treatment groups, the group differences in thickness of st. corneum were not significant. The insignificant differences in thickness of corneum were related

to the wide variation in its thickness within each sac within each dietary group. However, among different ruminal sacs, the differences in st. corneum were significant ($P<0.05$), being the thickest in both cranial and dorsal caudal blind sacs, followed by ventral caudal blind sac and the thinnest in both ventral and dorsal sacs (Table 2).

It is of interest to note that feeding PP diet decreased st. corneum thickness in all sacs, with exception for dorsal caudal blind sac, it increased thickness of corneum as compared to the control. However, st. corneum thickness in calves fed PF diet markedly increased in dorsal caudal blind sac, decreased in both cranial and ventral caudal blind sacs, and was not affected in both dorsal and ventral sacs (Table 2). In agreement with the present reduction in thickness of corneum in most ruminal sacs of calves fed PP diet, Abdel-Khalek (2000) found pronounced reduction in thickness of st. corneum as affected by feeding lambs on PP diet.

Lamina propria:

Lamina propria examined within each papilla was composed of a dense connective tissue, which was denser and more fibrous in calves fed control and PF diets than those found in calves fed PP diets. Lamina propria of all calves contained lymphatic vessels and blood capillaries, which were denser in PF calves (Fig. 8) than in PP (Fig. 9) and the control calves (Figs. 7). This finding may indicate more developed lamina propria in the calves fed PF and PP diets than that fed the control diet.

The cellular structures of the lamina propria were concentrated within the papillary bodies, which initiated as a result of invagination of the st. basal of lamina epithelialis. Similar finding was described by Khalil (1974) on sheep; Abdel-Khalek (1986 and 2000) on sheep and goats; Hemmoda (1981) on buffaloes and cows and Salama (1986) and Youssef (1992) on Egyptian buffaloes. The formation of these papillary bodies reduces the distance between the mucosa surface and absorptive sites and may increase the absorptive area. These papillary bodies were deeper and wider in papillae of both PP (Fig. 3) and control (Fig. 1) groups than in PF (Fig. 2) group.

Data in Table (2) show that thickness of lamina propria was affected significantly ($P<0.05$) by dietary treatment, being thicker in PP group than those in PF and control groups. Similar trend was observed by Abdel-Khalek (2000) in lambs fed PP diets as compared to those fed the control diet. Also, lamina propria was affected significantly ($P<0.05$) by ruminal sac, being the thickest in ventral sac, followed by both dorsal and ventral blind sacs, and the thinnest in cranial and dorsal sacs (Table 2). It is of interest to note that feeding calves on PP diet increased thickness of LP in dorsal, ventral and both dorsal and ventral caudal blind sacs. However, feeding calves on PF diet led to pronounced reduction in cranial and dorsal caudal blind sacs. This may suggest a reverse relationship between thickness of lamina epithelialis and that of lamina propria (Table 2) in different ruminal sacs of the same group. Similar trend was observed on adult cows by Hemmoda (1981).

Tunica submucosa:

The histological examination of all groups revealed that no distinction was observed between lamina propria of the papillae and their tunica submucosa.

Fig. (1): Ruminal papillae (P) of control calves showing lamina epithelialis mucosa (LEM), propria (LP), tunica submucosa (TSM) and papillary bodies (PB). (x40, E&H).

Fig. (2): Ruminal papillae (P) of calves fed PF diet showing lamina epithelialis mucosa (LEM), propria (LP), tunica submucosa (TSM) and papillary bodies (PB). (x40, E&H)

Fig. (3): Ruminal papillae (P) of calves fed PP diet showing lamina epithelialis mucosa (LEM) and propria (LP), tunica submucosa (TSM) and papillary bodies (PB). (x40, E&H).

Fig. (4): Serrated appearance of stratum corneum (SC) of calves fed control diet. (x200, H&E)

Fig. (5): Smooth appearance of stratum corneum (SC) in calves fed PF diet. (x200, H&E)

Fig. (6): Serrated appearance of stratum corneum (SC) in calves fed PP diet. (x200, H&E)

Fig. (7): The structure of lamina propria in control calves showing loose connective tissue containing a little of blood vessels (B), lymphatic vessels (L) and fibroblast cells (F). (x200, H&E)

Fig. (8): The structure of lamina propria in calves fed PF diet showing compact connective tissue rich in blood vessels (V), lymphatic vessels (L) and fibroblast cells (F). (x200, H&E)

Fig. (9): The structure of lamina propria in calves fed PP diet showing moderate connective tissue and its content of blood vessels (B), lymphatic vessels (L) and fibroblast cells (F). (x200, H&E)

Fig. (10): Tunica musculosa of control calves showing loose longitudinal (LM) and circular (CM) muscle bundles. (x40, H&E)

Fig. (11): Tunica musculosa of calves fed PF diet showing loose longitudinal (LM) and circular (CM) muscle bundles. (x40, H&E).

Fig. (12): Tunica musculosa of calves fed PP diet showing compact longitudinal (LM) and circular (CM) muscle bundles. (x40, H&E).

Consistent trend of differences in thickness of submucosa was achieved by feeding calves on PF and PP diets, being significantly (P<0.05) thicker in calves fed PF and PP diets than in those fed the control diet. However, tunica submucosa was significantly (P<0.05) thicker in both ventral and cranial sacs than in the other ruminal sacs (Table 3).

It is of interest to note that PF and PP diets had similar increase in submucosa thickness in dorsal and dorsal caudal blind sacs and decrease in ventral caudal blind sac as compared to the control diet. While submucosa thickness markedly increased in ventral sac of calves fed PP diet and in cranial sac of calves fed PF diet. In association with thickness of mucosa in different ruminal sac (Table 2), thickness of submucosa showed similar trend in all ruminal sacs, except for cranial and dorsal sacs, which showed submucosa thickness similar to the ventral sac (Table 3).

Table (3): Means and standard errors of thickness of tunica submucosa (mm) in different ruminal sacs of calves as affected by dietary treatments.

Group	Ruminal sac					Overall Mean
	Dorsal sac	Ventral sac	Cranial sac	Dorsal caudal blind	Ventral caudal blind	
Thickness of tunica submucosa (mm):						
Control	2.89 ± 0.09	3.42 ± 0.32	3.73 ± 0.44	2.58 ± 0.23	4.55 ± 0.39	3.43 ± 0.16^{B}
PF	3.29 ± 0.22	3.84 ± 0.31	6.71 ± 0.27	3.65 ± 0.21	2.32 ± 0.22	3.96 ± 0.20^{A}
PP	3.10 ± 0.10	7.13 ± 0.55	3.24 ± 0.14	3.76 ± 0.35	2.57 ± 0.24	3.96 ± 0.23^{A}
Overall mean	3.09 ± 0.09^{b}	4.79 ± 0.33^{a}	4.56 ± 0.29^{a}	3.33 ± 0.27^{b}	3.15 ± 0.22^{b}	
Thickness of circular muscle layer (mm):						
Control	4.35 ± 0.14	5.41 ± 0.17	5.49 ± 0.46	2.48 ± 0.10	4.66 ± 0.22	4.48 ± 0.16
PF	3.08 ± 0.14	5.00 ± 0.17	5.01 ± 0.32	2.87 ± 0.16	4.10 ± 0.14	4.01 ± 0.26
PP	3.67 ± 0.28	4.92 ± 0.38	4.10 ± 0.07	3.18 ± 0.21	4.71 ± 0.18	4.12 ± 0.21
Overall mean	3.70 ± 0.13^{b}	5.11 ± 0.15^{a}	4.87 ± 0.41^{a}	2.84 ± 0.10^{c}	4.94 ± 0.25^{a}	
Thickness of longitudinal muscle layer (mm):						
Control	5.13 ± 0.27	3.03 ± 0.13	3.89 ± 0.49	5.34 ± 0.40	3.10 ± 0.13	4.10 ± 0.18^{B}
PF	5.65 ± 0.35	9.15 ± 0.22	9.80 ± 0.31	6.01 ± 0.23	6.59 ± 0.32	7.43 ± 0.23^{A}
PP	8.11 ± 0.12	12.1 ± 0.52	6.80 ± 0.21	6.90 ± 0.93	3.19 ± 0.13	7.42 ± 0.43^{A}
Overall mean	6.30 ± 0.24^{b}	8.09 ± 0.59^{a}	6.83 ± 0.41^{b}	6.08 ± 0.35^{b}	4.29 ± 0.27^{c}	
Thickness of total musculosa (mm):						
Control	9.48 ± 0.21	8.44 ± 0.20	9.38 ± 0.91	7.84 ± 0.35	7.76 ± 0.30	8.57 ± .022^{B}
PF	8.73 ± 0.27	14.2 ± 0.25	17.8 ± 0.36	8.86 ± 0.28	10.7 ± 0.41	11.7 ± 0.46^{A}
PP	11.7 ± 0.16	16.9 ± 0.82	11.1 ± 0.23	10.1 ± 0.83	7.90 ± 0.19	11.5 ± 0.46^{A}
Overall mean	10.0 ± 0.17^{b}	13.2 ± 0.65^{a}	12.8 ± 0.69^{a}	8.92 ± 0.34^{c}	8.78 ± 0.19^{c}	

A & B and a, b & c: Means having different superscripts within the same row and column, respectively, are significantly different at P<0.05.

In general, the relationship between thickness of mucosa and submucosa was almost in positive pattern, increasing thickness of mucosa is in need to associate thicker submucosa to increase the absorptive capacity of the ruminal wall.

The present results concerning the increase in thickness of submucosa in calves fed PP diet come in line with those reported by Abdel-Khalek (2000) on histometric measures of tunica sub mucosa in rumen of lambs fed high level of undegradable protein diet.

Tunica musculosa:

In rumen of calves in all dietary groups, the muscle fiber layers of tunica musculosa were arranged mainly in two layers, an outer longitudinal and inner circular muscle layers. The connective tissue between the two layers and that penetrated them was almost in form of compacted muscle bundles in PP (Fig. 12) and in form of loose muscle bundles in PF (Fig. 11) and control calves (Fig. 10). Results of musculosa histometric revealed that both PF and PP diets significantly ($P<0.05$) increased the total thickness of tunica musculosa as compared to the control diet. The main effect of dietary treatments was observed in term of increasing ($P<0.05$) thickness of longitudinal than circular muscle layer (Table 3).

As affected by ruminal sac, musculosa was significantly ($P<0.05$) the thickest in both ventral and cranial sacs, followed by that in dorsal sac, and the thinnest in both dorsal and ventral caudal blind sacs. The significant increase in total thickness of musculosa in different ruminal sacs was mostly associated with significant increase in thickness of longitudinal and circular muscle layers. The pronounced increase in musculosa thickness was observed in dorsal and dorsal caudal blind sacs of calves fed PP diet and in ventral caudal blind sac of calves fed PF diet as well as in ventral and cranial sacs of calves fed PP and PF diets, respectively (Table 3).

The effect of dietary treatments, being significant ($P<0.05$) on thickness of longitudinal muscle layer and insignificant on circular one was accepted, since the outer longitudinal muscle layer is the main layer for muscular activity of the rumen in sheep and goats (Abdel-Khalek 1986). Development of the thickness of tunica musculosa may be related to the changes of the diet of lambs (Khalil, 1974) and it was affected by the nature of diet in calves (Tamate *et al.*, 1962). The present significant ($P<0.05$) increase in thickness of tunica musculosa as affected by PP diet was observed in lambs fed high level of undegradable protein diet (Abdel-Khalek, 2000).

Ruminal papillae:

The ruminal mucosa membrane is folded to form numerous papillae projected from the internal ruminal surface. These papillae were rather slender in shape and joined for approximately the first third of their length. Several authors reported different shape of papillae in lambs (Khalil, 1974), cows (Hemmode, 1981), sheep and goats (Abdel Khalek, 1986 and 2000), and Egyptian buffaloes (Salama, 1986 and Youssef, 1992).

Dietary effect (PF and PP diets) on papillary shape was not clear, where most papillae were slender in shape in calves of all groups (Figs. 1, 2 and 3). Generally, fillform papillae were found relatively more in case of feeding ruminants on CFM (Hemmoda, 1981).

Measurements and density of papillae:

Overall mean of papillary length in all ruminal sacs was affected significantly ($P<0.05$) by dietary treatment, being longer in PP than in PF group, but the differences between both groups and the control group were not significant. In the same line, papillae were significantly ($P<0.05$) longer in ventral, cranial and ventral caudal blind sacs than that in the dorsal caudal

blind sac. However, the dorsal sac showed significantly ($P<0.05$) the shortest papillae (Table 4).

Both PF and PP diets increased the papillary length in the ventral sac, being the longest and decreased them in the ventral caudal blind sac. However, dorsal sac showed the shortest papillae in calves of all groups (Table 4). These findings indicated longer papillae in the ventral region (both ventral and ventral caudal blind sacs) than in the dorsal region. Hemmoda (1981) reported similar variation between different regions of the rumen in adult cows and buffaloes.

Only ruminal sac significantly ($P<0.05$) affect overall papillary width, being the widest in the ventral sac, followed by ventral dorsal caudal blind, dorsal caudal blind and cranial sacs, respectively, while the dorsal sac showed the lowest values. Within each group, it was observed that feeding PF and PP diets increased length and decreased width of the papillae in the ventral sac (Table 4). The present investigation regard to the papillary length indicated wide variation in different individuals within the same group for different ruminal sacs and even in the same location. The differences between the ventral and dorsal region in papillary length may be related to concentration and accumulation of produced VFA ventrally in the floor of the rumen. The ruminal gases tend to accumulate dorsally and separate the roof from the rough ingesta, which is an important factor for the development of the ruminal papillae.

Warner *et al.* (1956) reported that the epithelial cells start in multiplication and migration as a papillary growth when a sufficient supply of VFA becomes available to the different regions of the rumen. The differences in papillary length in different sacs can be explained on the basis of microbial activity and production of VFA. In the Egyptian cattle the mean length of the ruminal papillae varied between 3.4-8.2 mm and may reach to 15.9 mm in the same location of the ventral region (Hemmoda, 1981). The corresponding lengths in the European cattle were 3-6 and 14.2 mm (Schnorr and Vollmerhaus, 1967) and in Egyptian buffaloes were 4.5 and 12.5 mm (Hemmoda, 1979).

This differences in papillary length in different dietary groups may be attributed to molar proportion of VFA and acetate: propionate: butyrate ratio produced via microbial fermentation of each diet. The earlier reports of Grummer (1988) and Schauff and Clark (1989) and the later one of Omer (1999) indicated that feeding ruminants on PF diets reduced molar proportion of butyrate compared to the control ration. However, total concentration of VFA did not change.

In case of feeding ruminants on PP diet, concentration of VFA significantly decreased (El-Reweny, 1999), while concentration of propionate increased and acetate decreased.

Overall mean of papillary density was significantly ($P<0.05$) lower in calves fed PF and PP diets than those fed the control diet. However, overall mean of density was significantly ($P<0.05$) the greatest in the dorsal, but the differences were not significant between ventral and dorsal sac as well as between dorsal and each of cranial and dorsal caudal blind sacs (Table 4).

The significant reduction in density of papillae in calves fed PP diet is in agreement with the results of Abdel-Khalek (2000), who found significant decrease in papillary density of lambs fed PP diet as compared to the control.

Table (4): Means and standard errors of papillary measures in different ruminal sacs of calves as affected by treatments.

Group	Ruminal sac					Overall
	Dorsal	Ventral	Cranial	Dorsal caudal blind	Ventral caudal blind	mean
Papillary length (mm):						
Control	1.97 ± 0.05	6.58 ± 0.65	15.51 ± 0.02	14.52 ± 1.80	21.04 ± 1.31	11.92 ± 1.8^{AB}
PF	2.10 ± 0.43	19.3 ± 3.80	14.77 ± 0.64	7.960 ± 0.39	10.61 ± 0.56	10.95 ± 1.6^{B}
PP	1.44 ± 0.09	19.6 ± 4.04	16.28 ± 0.56	13.61 ± 0.11	14.86 ± 0.68	13.17 ± 1.8^{A}
Overall mean	1.84 ± 1.4^{c}	15.2 ± 2.50^{a}	15.52 ± 0.34^{a}	12.03 ± 1.20^{b}	15.50 ± 1.30^{a}	
Papillary width (mm):						
Control	1.36 ± 0.03	2.02 ± 0.02	1.69 ± 0.10	1.75 ± 0.13	1.75 ± 0.12	1.72 ± 0.07
PF	1.31 ± 0.01	1.73 ± 0.12	1.43 ± 0.09	1.58 ± 0.06	1.72 ± 0.17	1.55 ± 0.05
PP	1.14 ± 0.12	1.72 ± 0.14	1.50 ± 0.10	1.50 ± 0.09	1.61 ± 1.17	1.50 ± 0.09
Overall mean	1.27 ± 0.06^{c}	1.82 ± 0.07^{a}	1.54 ± 0.07^{b}	1.61 ± 0.09^{b}	1.70 ± 0.08^{ab}	
Papillary density (No/cm^2):						
Control	166 ± 4.00	147 ± 6.53	153 ± 10.3	156 ± 1.30	103 ± 1.30	144.9 ± 8.00^{A}
PF	125 ± 12.0	140 ± 9.85	69 ± 36.1	84 ± 12.1	121 ± 10.0	107.9 ± 10.4^{B}
PP	104 ± 13.3	130 ± 8.60	92 ± 3.35	118 ± 7.60	81 ± 5.60	104.9 ± 11.3^{B}
Overall mean	132 ± 11.7^{a}	130 ± 8.61^{a}	105 ± 16.5^{b}	119 ± 11.1^{ab}	102 ± 7.80^{b}	

A & B and a, b & c: Means having different superscripts within the same row and column, respectively, are significantly different at P<0.05.

Total papillary surface area:

Total papillary surface area is depending on papillary dimensions (surface area of each papillae), density of papillae and inter-papillary area. Overall mean of surface area of each papilla was significantly (P<0.05) greater in calves fed PP diet than those fed PF and control diets, which did not differ significantly. However, overall mean of surface area per each papilla was significantly (P<0.05) the greatest in both ventral and ventral caudal blind sacs and the least in the dorsal sac (Table 5).

In calves fed PP or PF diets, values of surface area of each papilla markedly increased in ventral sac and decreased in both cranial and ventral caudal blind sacs. However, different trends of both diets were observed in dorsal and dorsal caudal blind sacs. This indicates pronounced effect of PF and PP on the ventral than the dorsal region of the rumen. Such effects were mainly related to the effect of dietary treatment on dimensions of the papillae located within each sac.

Papillary surface area per cm^2 was positively associated with surface area of each papillae rather than papillary density. This was reflected in nearly similar effect of dietary treatments on surface area of each papilla in different ruminal sacs (Table 5).

As affected by dietary treatments, only feeding calves on PF diet led to significant (P<0.05) reduction in overall mean of surface area per cm^2, however, PP diet showed insignificantly lower values compared with the control (Table 5). Overall mean of surface area per cm^2 was significantly

(P<0.05) the highest in ventral sac, followed by dorsal, ventral caudal blind and cranial sac. Meanwhile, the dorsal sac showed the lowest papillary surface area per cm^2 (Table 5).

Inter-papillary surface area per cm^2 showed negative relationship with papillary density and was significantly affected by dietary treatments, ruminal sac and their interaction. This relationship was indicated in all ruminal sacs and on the basis of overall mean of dietary treatment and ruminal sac (Table 5).

Table (5): Mean and standard error of papillary and inter-papillary sarface area in different ruminal sacs of calves as affected by dietary treatments.

Group	Ruminal sac					Overall Mean
	Dorsal	Ventral	Cranial	Dorsal caudal blind	Ventral caudal blind	
Surface area (mm^2)/papilla:						
Control	5.36 ± 0.15	26.58 ± 2.4	52.55 ± 3.23	51.01 ± 7.9	73.80 ±6.35	41.86 ±6.5^{B}
PF	5.49 ± 1.09	66.51 ± 4.2	41.91 ± 0.60	25.27 ± 2.0	36.27± 2.30	35.09± 5.4^{B}
PP	3.23 ± 0.21	65.25 ± 9.9	48.78 ± 3.46	56.85 ± 2.0	47.97± 5.77	44.42± 5.9^{A}
Overall mean	4.69±0.50^{c}	52.78 ± 7.3^{a}	47.75±2.10^{ab}	44.38 ± 5.5^{b}	52.68±6.01^{a}	
Surface area (mm^2)/cm^2:						
Control	690 ± 39	4043 ± 650	8445 ± 882	7921 ± 977	7420 ± 839	5664± 821^{A}
PF	696 ± 92	9274 ± 733	2921 ± 732	2135 ± 385	4380 ± 279	3882± 843^{B}
PP	341 ± 68	8495 ± 633	4484 ± 241	6723 ± 607	3856 ± 521	4780± 911^{A}
Overall mean	642 ± 95^{c}	7270 ± 932^{a}	5151 ± 916^{b}	5593 ± 967^{b}	5232 ± 625^{b}	
Inter-papillary surface area (mm^2)/cm^2:						
Control	51.6 ± 7.8	22.43 ± 9.0	30.3 ± 10.8	24.1 ± 11.0	50.1 ± 10.2	35.71± 4.9^{B}
PF	66.2 ± 6.3	33.9 ± 10.4	79.2 ± 9.20	66.3 ± 6.80	42.6 ± 9.20	57.65 ±5.6^{A}
PP	77.0 ± 8.7	39.4 ± 13.4	67.4 ± 3.80	18.3 ± 10.6	67.4 ± 6.90	53.68± 6.7^{A}
Overall mean	64.9 ± 5.0^{a}	31.9 ± 6.10^{b}	59.0 ± 8.50^{a}	36.3 ± 9.00^{b}	53.0± 6.00^{a}	
Total surface area (cm^2)/cm^2:						
Control	9.42 ± 0.38	40.65 ± 8.3	84.7 ± 8.71	79.5 ± 9.7	74.7 ± 8.3	56.9 ± 8.1^{A}
PF	7.63 ± 0.99	93.08 ± 7.2	30 0 ± 9.19	22.0 ± 3.7	44.2 ± 2.7	39.4 ± 8.3^{B}
PP	4.18 ± 0.59	85.34 ± 9.2	74.5 ± 2.38	67.4 ± 5.9	39.3 ± 5.1	48.3±9.0^{AB}
Overall mean	7.07± 1.01^{c}	73.02 ± 9.3^{a}	63.1 ± 9.08^{b}	56.3 ± 9.5^{b}	52.7 ± 6.2^{b}	

A & B and a, b & c: Means having different superscripts within the same row and column, respectively, are significantly different at P<0.05.

Total surface area per cm^2 of different ruminal sac mainly affected by surface area of papillae within each cm^2. So, total surface area/cm^2 showed the same trend of surface area of papillae/cm^2 as affected by dietary treatment and ruminal sacs. Differences in total surface of the ruminal mucosa by papillae reflected in different magnification rate in various ruminal sacs studied as affected by dietary treatment, being the highest within the ventral sac of calves fed PF and PP diets as compared to the control calves (Table 5).

As affected by dietary treatment, the overall magnification of the ruminal mucosa surface was lower in calves fed PF diet (39.4 times) than those fed PP (48.3 times) and control (56.9 times) diets. This indicated pronounced changes in total surface area per cm^2 as affecting by altering surface area of each papilla (length and width of each papilla).

It is of interest to suggest that the histological characteristics of the ruminal mucosa may be affected mainly by pattern of fermentation. Butyric and propionic acids seemed to have an important role in the development and growth of rumen (Kauffold *et al.* 1977 and Hemmoda, 1981). Moreover, Kauffold *et al.* (1977) found that propionate produced a proliferative effect on increasing thickness of lamina epithelialis, while acetic acid had antiprolierative effect. Dirkson *et al.* (1992) postulated that a well-proliferated ruminal mucosa could improve the energy supply by stabilizing the ruminal pH by providing a higher absorptive capacity for the VFA. In addition Henrksson and Habel (1961) reported that propionic acid found to stimulate epithelial growth and vaculation of st. garnulosum.

On the basis of the foregoing histogenesis and histometric characteristics of rumen in calves fed PF and PP diets, it could be conclude that the tested diets resulted in some changes in histogenesis of the ruminal wall, since increase fed PF and PP diets absorptive surface area of the interior surface of the rumen in calves fed PP diet was increased.

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دراسات غذائية وفسيولوجية علي عجول فريزيان مغذاة علـي دهـن محمـى و بروتين محمى:

٦- الخصائص الهستولوجية والهستومترية للكرش.

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أجريت هذه الدراسة في محطة بحوث الإنتاج الحيواني بسخا واستخدم في هذه الدراسة ١٨ عجل فريزيان رضيع في الشهر الأول من العمر واستمرت التجربة حتى الشهر الثامن من العمر، ووضعت هذه العجول في ثلاثة مجاميع متشابهه ، المجموعة الأولي مجموعة مقارنة (كنترول) ، والمجموعة الثانية غذيت علي العليقة المركزة المحتوية علي ٥% دهون محميه ، والمجموعة الثالثة غذيت علي العليقة المركزة المعاملة بالفورمالدهيد ١% لحماية البروتين وكان الهدف من هذه الدراسة معرفه تأثير كل من البروتينات المحمية والدهون المحمية علي الخصائص الهستولوجية والهستومترية للكرش ولذلك تم ذبح عدد ٣ عجول من كل مجموعة في نهاية التجربة (عمر ٨ شهور) وتم عمل قطاعات هستولوجية من مناطق الكرش المختلفة لقياس سمك الطبقات المختلفة لجدار الكرش وكذلك ابعاد وكثافة الحلمات الكرشية لحساب مسطح الأمتصاص للحلمات الكرشية/سم٢ من سطح الكرش. وكانت النتائج المتحصل عليها كالتالي:

١- كان سمك الطبقة المخاطية للكرش أكبر معنويا فى العجول المغذاة على البروتين المحمى من تلك المغذاة على الدهون المحمية والعليقة المقارنة.

٢- لم يختلف سمك الطبقة الطلائية المخاطية معنويا فى العجول المغذاة على البروتين المحمى والدهن المحمى عن العجول المقارنة ولكن كان السمك أعلى معنويا فى المعجول المغذاة على الدهون المحمية عن تلك المغذاة على البروتين المحمى (٣٠ مم مقابل ٢٧مم).

٣- كان سمك الطبقة القرنية للكرش أكبر فى العجول المقارنة (٤٤,٣ ميكرون) عن تلك المغذاة على الدهون المحمية (٤٢,٩ ميكرون) والبروتين المحمى (٤٣,٢ميكرون) ولكن الفروق كانت غير معنوية.

٤- زاد سمك طبقة النسيج الضام المخاطى معنويا فى العجول المغذاة على البروتين المحمى عن تلك المغذاة على الدهون المحمية والعليقة المقارنة (١,٢ مقابل ٠,٩٦ و١,٠٢ ميكرون ، على الترتيب).

٥- زاد سمك الطبقة تحت المخاطية معنويا فى العجول المغذاة على الدهون والبروتينات المحمية عن تلك فى المجموعة المقارنة.

٦- لم يختلف سمك العضلات الدائرية للطبقة العضلية للكرش معنويا ولكن اختلف سمك العضلات الطولية معنويا بين المجموعات وبالتالى كان سمك الطبقة العضلية الكلية أكبر فى العجول المغذاة على الدهون المحمية والبروتين المحمى عن تلك فى المجموعة المقارنة.

٧- كان سمك الطبقات المختلفة لجدار الكرش مختلف معنويا فى المناطق المختلفة للكرش وكان سمك معظم الطبقات أعلى معنويا فى الكيس البطنى للكرش لعجول جميع المجموعات.

٨- لم يختلف طول الحلمات فى كرش العجول المغذاة على الدهون المحمية والبروتين المحمى عن المجموعة المقارنة ولكن الحلمات كانت أطول معنويا فى العجول المغذاة على الدهون المحمية عن تلك المغذاة على البروتين المحمى.

٩- لم يختلف عرض الحلمات معنويا بين المجموعات (١,٥٥، ١,٥ و ١,٧٢ مم) فى العجول المغذاة على الدهون المحمية والبروتين المحمى والمقارنة على الترتيب.

١٠ - كانت الحلمات الكرشية أقل كثافة معنويا فى العجول المغذاة على الدهون المحمية والبروتين المحمى عن المجموعة المقارنة. وكان متوسط مساحة كل حلمة أقل معنويا فى العجول المغذاة على الدهون المحمية عن تلك المغذاة على البروتين المحمى والعليقة المقارنة.

١١ -كانت مساحة الحلمات لكل سم٢ من سطح الكرش الداخلى منخفضة معنويا فى العجول المغذاة على الدهون المحمية عن المجموعة المقارنة بينما لم تختلف العجول المغذاة على البروتين المحمى معنويا عن المجموعة المقارنة.

١٢ - أظهرت المساحة بين الحلمات لكل سم٢ من سطح الكرش قيم عكسية مع كثافة الحلمات وكانت أكبر معنويا فى العجول المغذاة على الدهون المحمية والبروتين المحمى عن المجموعة المقارنة. بينما كانت المساحة الكلية (مجموع المساحة للحلمات وبين الحلمات) لكل سم٢ من سطح الكرش منخفضة معنويا فى العجول المغذاة على الدهون المحمية عن تلك المغذاة على البروتين المحمى والعليقة المقارنة.

وبناء على نتائج الدراسة المقدمه فإنه من الناحية الغذائية يمكن إستنتاج أن تغذية العجول على الدهون المحمية أو البروتين المحمى خلال فترة الرضاعة والفترة المبكرة بعد الرضاعة لها تأثيرات إيجابية على بعض الخصائص الهستولوجية والهستومترية للكرش أثناء التطور.