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Creep Feeding Supplemented with Roughages Improve Rumen Morphology in Pre-Weaning Goat Kids

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NNH did experimental work. BZ and ATK helped in data analysis. TK and EAW helped in writing the manuscript. MAK supervised the experiments.

Key words
Rumen, Creep feeding, Goat kids, Roughages.

ABSTRACT

Boer crossbred goats (n=48) single born kids, seven-day-old (live weight 4.4±0.09kg) were divided into 3 treatment groups, each having 8 females and 8 males. The kids from first treatment group had free access to creep feed with roughage (CFR), second group had free access to creep feed without roughage (CF) while last group (control) had access to their does' milk (DM) only. All kids had access to their dam's milk. The kids were weaned at day 84 and two kids from each treatment group were sacrificed. The results showed that the rumen morphological characteristics, papillary surface area and keratinized layer of CFR (4.15mm^2, 50.54µm), were not significantly different from CF (3.81mm^2, 49.26µm) ($P>0.05$) but it was significantly different from kids in DM group (1.17mm^2, 26.36µm) ($P<0.05$). The rumen papillary surface area and keratinized layer were also significantly different between CF and DM ($P<0.05$). The rumen of kids from CFR group had higher sub-mucosa layer thickness (619.8µm), muscle layer thickness (1155.7µm) and rumen wall thickness (1775.4µm) than the rumen of kid in CFR and DM groups ($P<0.05$). The total surface area of rumen of kids from CFR and CF groups was not significantly different ($P>0.05$), while total surface area of rumen of both creep fed groups was significantly greater than DM group. The rumen tissue weight of creep-fed kids was 17% greater than those of milk fed kids. Overall, the present study showed that the creep feed supplementation to nursing goat kids improve rumen morphology.

INTRODUCTION

Creep feeding is the supplementation of goat kids with concentrate supplement or creep grazing with high quality forage. Usually, creep allow kids to feed on or graze supplementation at will access while restrict access of larger mature goats. Creep feedings provide the potential to improve performance through suckling goat kids under limited forage mass and quality conditions. The conditions of forage and the time of year can influence the effectiveness of creep supplementation (Cabrera *et al*., 2013; Lardy and Maddock, 2007; Reis *et al*., 2015). Development of Rumen is characterized by incredible increase in volume, mass and surface area. Tissue growth is accompanied by a differentiation, the outcomes of which is physical changes such as papillary development and increased keratinization (Tamate *et al*., 1962). Variations in the form and the type of nutrients which are delivered to the gastrointestinal tract might change cellular proliferation, total nutrient usage by the gut and eventually, the availability of nutrient to support growth in young ruminants (Bhatt *et al*., 2009; Coverdale *et al*., 2004; Norouzian *et al*., 2011).

Although growth of the rumen generally is related to age or body weight, the best predicator of rumen weight may be intake of solid food. It has been known that the fore-stomach of the calf fed milk only develops very slowly while feeding hay and/or grain caused rapid development (Foley *et al*., 2009). However, forage digestion by microorganisms does not offer enough concentration of rumen volatile fatty acid, especially butyrate required for optimal papillae development (Norouzian *et al*., 2011). Fermentation of concentrate provides the necessary butyrate to stimulate papillae development, but these feeds may cause keratinization of the papillae in calves and lambs (Beiranvand *et al*., 2014; Raghuvansi *et al*., 2007; Wang *et al*., 201; Khan *et al*., 2016).

The rumen is of utmost important as the fermentation and absorptive organ and for synthesis of microbial protein. The development of the rumen is essential for successful weaning and good growth rate after weaning (Beiranvand *et al*., 2014; Connor *et al*., 2013). After weaning, the kids totally rely on the dry feed for their

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nutrient needs and therefore, the kids should consume adequate amount of solid food to develop their rumens before they are weaned. Supplementation of creep feed to the nursing kids can enhance rumen development so that at weaning their rumens developed already. It is hypothesized that the supplementation of creep feed (with or without roughage) can improve rumen morphology as compared to the milk fed kids. The objective of this study was to examine the influence of supplementation of creep feed on the development of rumen.

MATERIALS AND METHODS

Experimental animals and design

The experimental goat kids were kept at commercial farm (KLAS Farm) located at Melaka State, Peninsular Malaysia. Single born, seven-day-old (n=48) Boer crossbred kids were randomly allotted into treatment groups of three by using Research Randomizer software. Each group was comprised of eight males and eight female kids containing an average weight of 4.4±0.09 kg. The kids in all 3 groups were allowed to suckle their mother's milk until they were weaned at day 84. During the experimental period, kids were allowed to stay with their does from 6pm to 8am and from 12 pm to 2 pm. The treatment groups were: (a) Treatment: Kids present in this group were fed dam's milk + creep feed having roughage (alfalfa hay (*Medicago sativa*), chopped to a length of between 1 and 2cm) (CFR), (b) Treatment 2: Kids present in this group were fed dam's milk + creep feed without roughage (CF) and (c) Treatment 3: Kids in this group had access only to dam's milk (DM). DM was the fundamental feed for all three groups. Therefore, treatment 3 (DM) was the control group in this study.

Feeding the kids

The only born kids were placed together with their doe in individual pens (4 × 5 feet) and had free access to their mother's milk, except during feeding time. At the feeding time, the kids from the three groups were kept separated from their mothers in their feeding pens (12 × 12 feet). The treatment groups CFR and CF were fed according to allotted feedings (Table I) *i.e.* twice a day (0800 – 1200 h and 1400 – 1800 h while the control group, DM was provided by water only. Does were fed twice a day; from 0800 to 1200 h and from 1400 h to 1800 h. The left over feed from the does' feeding troughs were removed at the end of their feeding time before the kids were allowed to their doe's pen. The kids from CFR and CF were fed creep feed (5% of body weight) on the basis of dry matter. Feed were renewed on the daily basis. Clean water was given to all the groups during this study.

Table I.- The feeding program of the experimental groups; CFR, CF and DM.

Time (h)	CFR	CF	DM
0800 – 1200	Creep feeding	Creep feeding	Water only
1200 – 1400	With mother	With mother	With mother
1400 – 1800	Creep feeding	Creep feeding	Water only
1800 – 0800	With mother	With mother	With mother

CFR, kids fed creep feed containing roughage; CF, kids fed creep feed without roughage; DM, kids fed dam's milk only.

Table II.- Ration composition and chemical analysis of creep feeds fed to T1 (CFR) and T2 (CF).

Creep feed components	Feed type	
	T1 (CFR)	T2 (CF)
Ration composition (%)		
Soya hull	32.0	34.0
Corn	33.0	37.0
Palm kernal cake	17.0	22.0
Alfalfa hay	11.0	-
Molasses	5.0	5.0
Ammonium chloride	0.5	0.5
Sodium chloride	0.5	0.5
Calcium carbonate	1.0	1.0
Chemical composition		
Crude protein (g/100g)	14.0	14.2
Crude fat (g/100g)	5.0	4.4
Carbohydrate (g/100g)	64.2	62.7
Ash (g/100g)	4.6	5.5
Moisture (g/100g)	12.2	13.2
Metabolizable energy (kcal/kg)*	3162	3066
Crude fiber (g/100g)	5.2	0.3

% Total carbohydrate = 100 – (%Ash + %Moisture + % Protein + %Fat).
*, calculation by Factor.
For abbreviations, see Table I.

Creep feed

Creep feeds (mash form) were formulated on the basis of the nutritional requirements of goats by using locally available raw ingredients. For the chemical analysis of crude fat, crude protein, crude fiber, carbohydrate, moisture, ash, metabolizable energy, the samples of creep feed were sent to the reference laboratory (Unipeq, Sdn. Bhd. Kuala Lumpur, Malaysia). The compositions of feed and the chemical analysis of formulated creep feeds are shown in Table II.

Anatomical techniques and measurements

During slaughter, the rumen, reticulum, omasum and abomasum were separated and weighed individually. Each compartment was cleaned, emptied, washed, drained and weighed. The volume of different stomach compartments (reticulum, rumen, abomasum and omasum) was measured by using the water filling method. One opening of the compartment was clamped with a forceps, and water was filled though the other opening following the method described by Ward (2008).

Histological techniques and measurements

Three rumen tissue samples from each slaughtered kid (1 cm^2) were obtained from identical sites of the *anterium ruminis,* ventral ruminal sac and dorsal sac, caudo-dorsal sac, caudo-ventral sac and left pillar (n=18) within 30 min of death as described by Norouzian *et al*. (2011).

Rumen tissue measurements

Five papillae were selected from each slide. The length, width and thickness of the layers; keratin, sub-mucosa, muscularis and rumen wall were determined. Morphometric measurements were performed using an Olympus Microscope (BX 53). For measuring the parameters, the microscopic image was taken in a Olympus DP 72 digital camera while image measurements.

Surface area of papillae

The surface area of each papillae was determined using the following formula (Zitnan *et al*., 2005):

Surface area of papillae (mm^2) = Length × Width × 2

Papillary length was defined as the distance from the tip to the base of the papilla and the papillary width was the average width of the base, middle and tip of the papillae (Norouzian *et al*., 2011). All these measurements were carried out under the same magnification (× 4) (Olympus BX 53 Microscope).

The density of papillae for each 1 cm^2 of the rumen samples was determined by calculating the total number of papillae in the field of a Stereo microscope (Olympus SZ61) at a magnification of (0.67×) as described by Klein *et al*. (1987). The average width of the papillae was average width of the base, middle and tip of the papillae. The inner papillary (the area covered by papillae per cm^2) was computed by multiplying the average width of papillae with the density of the papillae (number of papillae per cm^2).

Total papillary surface area per cm^2 was computed by multiplying the average surface area of papillae with density of papillae per cm^2 of that rumen as follows:

The total absorption surface area per 1cm^2 of each rumen tissue was calculated by summation of total inner papillary area and total papillary surface area.

Statistical analysis

Data collected from individual characteristics of rumen development were analyzed by using one-way ANOVA, SPSS (version 19). Difference between means was tested for significance according to Tukey Test with the significant level of P<0.05. Descriptive statistics was used for the reticulum, omasum and abomasum measurements.

Table III.- Papillae length, papillae width, and surface area of papillae, keratinized layer, sub-mucosa layer, muscle layer and rumen wall thickness of Boer goat kids at 12 weeks of age. The values are Mean±SEM.

Parameters	**n**	**CFR**	**CF**	**DM**
Papillae length (µm)	60	3121.4 ± 148.9 [a]	3690.7 ±124.7 [b]	1330.5 ± 109.8 [c]
Papillae width (µm)	60	634.0± 31.7 [a]	496.3 ± 21.5 [b]	418.0 ± 16.9 [c]
Papillae surface area (mm^2)	60	4.15 ± 0.38 [a]	3.81 ± 0.21 [ab]	1.17 ± 0.12 [c]
Keratinized thickness (µm)	180	50.94± 2.38 [a]	49.26.3 ±2.59 [ab]	26.36 ± 1.22 [c]
Mucosa thickness (µm)	36	619.8± 30.5 [a]	453.8 ± 34.8 [b]	439.4 ± 27.1 [bc]
Muscularis thickness (µm)	36	1155.7 ± 35.0 [a]	1009.9 ± 45.8 [b]	823.0 ± 40.1 [c]
Rumen wall thickness (µm)	36	1775.4 ± 49.4 [a]	1463.7 ± 65.4 [b]	1262.3 ± 60.5 [c]

n, number of samples from each group (Number of animal used in each group =2; not identified sex). [*a,b,c], values with different superscripts in the same row differ significantly (P<0.05).

RESULTS

Rumen morphometrical characteristics

The rumen papillae of CF group were significantly longer (P<0.05) than the papillae of other groups (CFR and DM) whereas the papillae of DM group were the shortest (P<0.05) (Table III). It was found that rumen papillae of CFR group were broader and had tongue like appearance with rough surface. In the CF group, the papillae were round, tall and had smooth surface whereas papillae in the DM group were small, short and smooth surface. However, the significantly broader papillae width was noted in kids from CFR group (P<0.05) than the other treatment groups. The kids of DM groups had the narrowest papillae width (Table III, Fig. 1). The papillae surface area and keratin layer thickness between CFR and CF groups were not significantly different (P>0.05) while the creep fed groups had significantly increased papillae surface area and thicker keratin layer than milk fed kids (P<0.05). The kids from CFR groups appeared to have the thickest rumen mucosa layer (P<0.05) but there was no significant difference on the thickness of rumen mucosa in kids from CF group and DM group (P>0.05) (Fig. 2).

Fig. 1. Caudo-ventral sac of the rumen of experimental kids (12 weeks of age) showing the density of the rumen papillae per cm^2 under stereo microscope (0.67×). A, rumen of kids with dam's milk alone; B, rumen of kids fed milk + creep feed without roughage; C, rumen of kids fed milk + creep feed containing roughage.

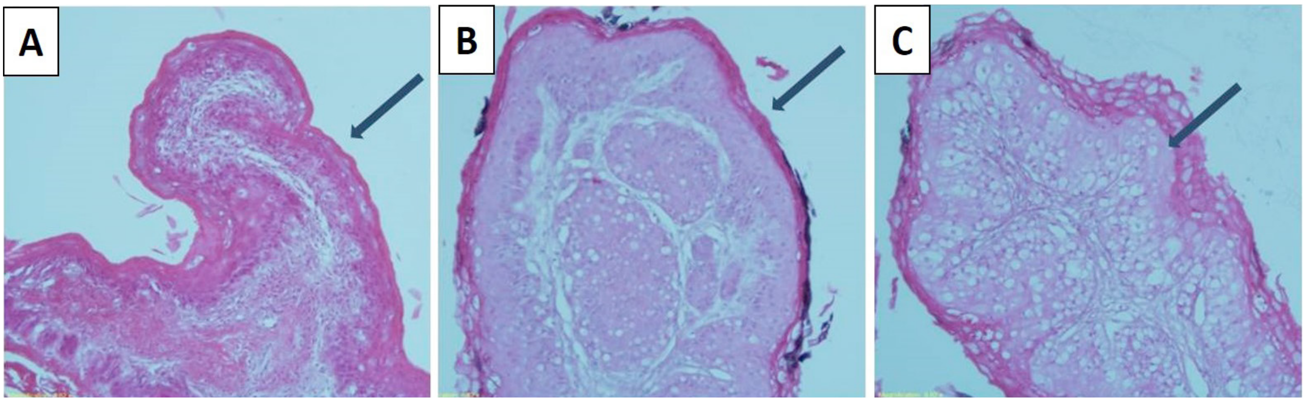

Fig. 2. Rumen papillae from ventral sacs, arrows show keratin layer of papillae in A, DM; B, CF and C, CFR. The keratin layer of DM group is very thin, CF group was thick and compact and CFR group was thick and had vacuolated cells (× 20; bar = 500 µm).

Table IV.- Papillae density per cm^2, total papillae area, total inner papillary area, and total surface area per cm^2 of CFR, CF and DM kids at 12 week of age.

	n	CFR	CF	DM
Papillae density (per cm^2)	36	109.39 ± 3.49^{a}	109.64 ± 2.60^{ab}	180.17 ± 5.67^{c}
Total papillae surface area (mm^2/cm^2)	36	516.16 ± 30.06^{a}	472.16 ± 27.24^{ab}	243.51 ± 22.98^{c}
Total inner papillary area (mm)	36	73.67 ± 2.93^{a}	60.14± 3.37^{b}	81.07 ± 3.42^{ac}
Total surface area (mm^2/cm^2)	36	594.57 ± 31.65^{a}	533.03 ± 30.03^{ab}	322.08 ± 23.83^{c}

n, number of samples from each group (Number of animal used in each group =2; not identified sex). *^{a,b,c}, values with different superscripts in the same row differ significantly (P<0.05).

Rumen development

The kids fed dam's milk had significantly highest density of papillae per cm^2 (P<0.05) as compared to creep fed kids. However, no significant difference on the papillae density between kids of CFR group and CF group was noted. Total papillary surface area per cm^2 of rumen was greatest in kids of CFR group but it was not significantly different from kids of CF group (P>0.05). But the kids fed dam's milk had significantly smallest total papillary surface area per cm^2 (P<0.05) as compared to kids from creep fed groups (Fig. 2). The total rumen surface area of kids in the CFR group and CF group was similar (P>0.05) but they both had significantly larger total rumen surface area of kids than in DM group (Table IV).

Anatomical development of rumen

The fresh tissue weight of rumen, reticulum, omasum and abomasum of CFR group fresh tissue of the whole stomach were 67%, 8%, 6%, 19% and for CF group were 67%, 10%, 6%, 17% and for DM group were 50%, 14%, 8%, 28%, respectively (Table IV). The creep fed kids had the highest percentage of rumen weight whereas the milk fed kids had the lowest. However, no difference was observed in rumen tissue weight between CFR group and CF group. The milk fed kids had higher percentage of reticulum, omasum and abomasum as compared to the creep fed kids. The volume capacity of rumen, reticulum, omasun and abomasum of CFR group were 81%, 5%, 1%, 13% and for the CF group were 75%, 16%, 1%, 8% and for the DM group were 79%, 8%, 1%, 12%, respectively. The volume capacity of the rumen of kids of CFR group was the highest as compared to the CF group and DM group (Table V).

Table V.- Mean of the fresh rumen weight, rumen volume capacity, fresh reticulum weight, reticulum volume capacity, fresh omasum weight, omasum volume capacity, fresh abomasum weight and abomasum volume capacity of the three experimental groups (CFR, CF and DM).

	CFR	CF	DM
Fresh rumen weight (g)	306	291	102
Rumen volume capacity (cc)	2825	2425	1912
Fresh reticulum weight (g)	39	43	29
Reticulum volume capacity (cc)	164	199.0	187.0
Fresh omasum weight (g)	29	25	17.5
Omasum volume capacity (cc)	41.5	30.5	33.5
Fresh Abomasum weight (g)	90.0	73.5	57.5
Abomasum volume capacity (cc)	457.5	261.0	286.0

DISCUSSION

The rumen papillae of kids fed milk only were the least developed in length, width, surface area and total surface area per cm^2 as compared to those fed creep feed containing roughage and creep feed without roughage. This is most likely attributed to the nature of its feed, *i.e.* milk. When kids suckle milk, it passes through the rumino-reticular groove to abomasum, thus little or no fermentation can occur in the rumen. Dry feed consumption has been reported to be the primary factor of dairy calf's rumen development *i.e.* papillae length, papillae width, total surface area of the papillae per cm^2 (Laborde, 2008). When the lambs were given dietary supplementation with essential oils, it would go to the rumen first and consequently microbial digestion consequently increases the volatile fatty acids production (Vasta *et al*., 2013). Similar findings has also been observed in pigs supplemented with cereal and enzyme (Pauly *et al*., 2011). The VFAs, especially propionate and butyrate are main stimulators for rumen papillae development. The lambs fed with pellets had significantly wider and longer papillae than those fed milk replacer alone at 12 weeks of age (Lane *et al*., 2000).

VFA infusion could increase papillary length but the increase in length was not as great as that during normal rumen development of lambs indicating that VFA appear to stimulate some aspects of rumen development and might not be responsible for all morphological and metabolic changes between birth and maturity in the rumen of animals consuming solid feed (Lane *et al*., 2000). The width of the rumen papillae of CFR group was broader than CF group. This finding agrees with Suarez *et al*. (2007) who found that the width of the rumen papillae of the calves fed total mixed ration (90% starter + 10% alfalfa hay) had broader papillae as compared to calved fed (100% starter) and to calves fed (starter + free choice alfalfa hay). Surface area of papillae and total surface area per cm^2 were not significantly different (P>0.05) between CFR and CF. This finding agrees with Norouzian *et al*. (2011) who found that there was no effect of including forage in the starter diet on height and width of rumen papillae for lambs fed a starter diet containing alfalfa (7 and 15%) or the starter diet only.

In the present study, the keratin layer of rumen papillae, of kid in the CFR and CF groups were significantly thicker than kids fed does' milk only. The keratin layer thickness depends on the VFA production of the feed. In this experiment, keratin layer of DM group was thinner than other groups because kids from DM group relied only on the milk as VFA production of the milk was lesser than VFA production of kids receiving creep feed plus milk. There was no significant difference (P>0.05) of keratin layer thickness between CFR and CF groups. Norouzian *et al*. (2011) found that the thickness of keratin layer in lambs fed with starter supplemented with 15% alfalfa hay was significantly better (P<0.05) as compared to those getting starter only while no significant difference was found in other group fed with 7.5% alfalfa hay. In our study, 11% of the roughage content in creep feed also did not influence the keratin layer thickness (Table III).

In the present study, kids fed does' milk only had the highest papillae density per cm^2 compared to the two creep-fed groups. This is most likely due to their smaller size of papillae. The rumen of DM group comprised of small and thin (418.0 µm) papillae compared to the other treatments (CFR and CF) which had broader (634 µm, 496.3µm) papillae respectively. Our results agrees with Norouzian *et al*. (2011) which showed that there was no effect of forage inclusion in the starter diet (7.5% or 15%) on papillae density. The similarity of the density of rumen papillae in CFR and CF groups indicate that creep feeding (with or without roughage) stimulate the papillae development. Similar results in calves were reported by Suarez *et al*. (2007) in which the calves fed calf starter 90% and 10% ground alfalfa had the thickest sub-mucosal layers than the calves fed free choice alfalfa hay and calf starter alone. Castillejos *et al*. (2006) have stated that the high sub-mucosal thickness of the calves getting total mixed diet might be attributed to high acidity of rumen which lead to high blood flow to tissues.

In this study, muscle layer thickness and ruminal wall thickness of the three treatment groups were significantly differ from each other, CFR group being the thickest muscle layer. This might be due to the physical structure of the feed, stimulating physically the rumen wall. The feed of CFR group contained roughage which stimulates the rumen more than CF and DM groups. This finding is in accordance with Norouzian *et al*. (2011), where lambs fed feed containing alfalfa hay had the thickest rumen muscle layer. Huge particle size of roughage, high content of effective fiber and increased bulk of forages or high fiber sources increase rumen wall by physical stimulation, and subsequently increase rumen motility, muscularization and rumen volume. The fresh rumen tissue weight of CF and CFR groups was greater than that of DM group while the volume capacity of the rumen of kids in CFR group was the highest as compared to CF group and DM group which may be due to the feed containing roughages that increased the bulk of the rumen. The results of the present study demonstrated that creep feed supplementation with roughages to nursing kids improve rumen morphology.

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Statement of animal rights

The use of the all the animals in this study was approved by the Animal Ethic Committee, Faculty of Veterinary Medicine, Universiti Malaysia Kelantan, Malaysia.

Statement of conflict of interest

Authors declare that they have no conflict of interest.

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