TCTP Promotes Hu Sheep Rumen Epithelial Development before Weaning

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Research

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Abstract

Background

Hu Sheep is a world precious breed of sheep. Rumen is the most important digestive organ for ruminant animals. A better understanding of the molecular mechanisms involved in rumen development help us design better strategies to improve the production of sheep. Translationally controlled tumor protein (TCTP) is a highly conserved protein that involves various cellular processes. However, its role in rumen epithelium development remains unknown.

Results

TCTP was expressed in stratum basale, stratum spinosum and stratum granulosum of rumen epithelium. TCTP mRNA expression was extremely high on the day of birth, it then significantly decreased on day 15 and gradually increased until day 45 of age. TCTP protein expression remained in a relative low level from day 0 to day 15 of age, it then significantly increased on day 30 and gradually decreased until day 60. The ratio of Ki67 positive cell in stratum basale cell followed the similar pattern as the expression of TCTP. The papillae length decreased at first 15 days of postnatal life. Thereafter, the papilla undergo a period of growth from 538.1±17.3μm to 2211.1±56.6μm until 60 days of age. The phosphorylation of AKT is the highest on day 15 of age, then decreased until day 45. The ratio of acetate:propionate in rumen fluid decreased from day 30 to day 60 of age.

Conclusions

TCTP participates in rumen papillae growth by promoting rumen stratum basale cell proliferation. We suggest that the translation of TCTP mRNA is regulated by AKT phosphorylation and the development of rumen papillae is associated with acetate:propionate ratio.

Background

Hu Sheep is a world precious breed of sheep, which is known for its high fertility and tolerance of crude feed[1]. Nowadays, following the growing need for mutton, Hu Sheep has been breed from lambskin sheep to mutton sheep. Rumen is the most important digestive organ for ruminant animals, which determines the production of ruminants[2]. Rumen epithelia is responsible for up to 70% of the energetic needs of mature animals [3]. The papillae length of the rumen is considered as the key factor for the evaluation of rumen development[4]. The proliferation and growth of the rumen squamous epithelium not only promotes the growth of papillae length and width, but also increases the thickness of the interior rumen wall[5]. Current understanding to the sheep rumen papillae development before weaning is limited. Hence, we tried to investigate the pattern of rumen epithelia development before weaning and potential molecular signals involved in this progress.

TCTP is a highly conserved protein that participates in various cellular processes, including cell growth, proliferation, and metabolism[6, 7]. Emerging studies have found TCTP a key regulator in organ development, and its role in growth control is conserved in many eukaryotes[8-10]. However, It remains unclear whether TCTP exerts such function in sheep rumen epithelium. Given the importance of TCTP in organ growth, we were promoted to determine the expression and function of TCTP in sheep rumen epithelia development before weaning.

In this study, we first firmly established that TCTP expressed throughout the weaning stage of Hu Sheep. Then we described the histological and morphological changes of rumen papillae from birth to weaning. We also found rumen epithelia basal cell proliferation associated with TCTP expression. Furthermore, we found TCTP translation associated with AKT phosphorylation and the ratio of acetic acid/propionic acid. These findings raise the possibility that TCTP promoted rumen epithelia basal cell proliferation, which is regulated by PI3K/AKT signaling and the ratio of acetic acid/propionic acid might regulate the growth of rumen papillae.

Materials And Methods

Animal preparation

Twenty-five healthy male Hu Sheep of were randomly selected and sacrificed either at day 0, day15, day 30, day 45 or day 60 of age with 5 sheep in each age. The rumen tissue were fixed in 4% PFA for 48 hours. Rumen epithelium was isolated and frozen in liquid nitrogen. Experimental protocols for animal research were approved by the Institutional Animal Care and Use Committees at the Zhejiang Academy of Agricultural Sciences.

RNA extraction and real-time PCR

The rumen epithelium tissue were carefully isolated and quickly cleaned. The samples were then frozen and ground to homogeneity in liquid nitrogen. Total RNA was extracted from 0.5 g samples of frozen tissue using the RNAiso Reagent (TaKaRa, Dalian, China) according to the manufacturer's instructions. Then, 2 μg of isolated RNA from each sample was reverse transcribed into cDNA using SuperScript III reverse transcriptase (Thermo Fisher Scientific). Quantitative PCR was carried out to determine the mRNA levels of TCTP with iTaq Universal SYBR Green Supermix (Bio-Rad, Hercules, CA, USA) in accordance with the manufacturer's protocols. Then, the relative level of expression of each target gene was calculated using the $2^{-\Delta\Delta}$Ct method. Target genes were normalized against the 18s rRNA before further analysis. All the primers are listed in Table 1.

Table 1 Primer sequences for sheep *TPT1*

Name	Sequence, 5’–3’	
	Forward	Reverse
TPT1	GGGAAACTTGAAGAACAGAGAC	ACACCATCCTCACGGTAGTC
18s rRNA	CACACGGACAGGATTGACAGATT	GAGCCAGTCAGTGTAGCGCG

Histology analysis and immunohistochemistry

For histological analysis, the rumen tissues kept in PFA were dehydrated with alcohol and embedded in paraffin, sectioned, and stained with hematoxylin and eosin (H&E), and the sections were observed under a microscope (Nikon, NY, USA). The length of rumen papillae were measured by using imageJ (version 1.44).

Paraffin-embedded rumen samples sectioned at 5 μm and mounted on slides for immuno-histochemical staining. The nonspecific binding of primary antibodies was blocked by incubation with PBS containing 5% BSA and 0.2% Triton X-100 for 1 h. Incubation was carried out overnight at 4℃ with rabbit polyclonal antibodies to TCTP (ab37506, abcam, dilution 1:200), AKT (#9272, cell signaling, dilution 1:200) and ki67 (ab15580, abcam, dilution 1:500). The immunostaining was visualized using the Elite SABC kit (boster biological technology, Wuhan, Hunan, China) and DAB visualization kit (boster biological technology, Wuhan, Hunan, China). For the negative controls, primary antibodies were replaced with PBS. The sections were counter-stained with hematoxylin to identify the cell structure and type. Three researchers blinded as to slide identity evaluated the samples. Staining intensity was graded as follows: -, no staining; +/-, a few staining; +, faint staining; ++, moderate staining; and +++, strong staining.

Protein extraction and Western blot

For protein extraction, rumen epithelia tissue were lysed in Laemmli sample buffer (SDS sample buffer with 2-mercaptoethanol). Protein concentrations were determined using a standard bicinchoninic acid assay. Prior to electrophoresis, samples were heated for 5 min at 95℃, and 20 μg of protein was loaded per lane. Samples were subjected to sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis under standard conditions and were transferred onto polyvinylidene difluoride (PVDF) membrane in a buffer containing 0.2 mol/L glycine, 25mM Tris, and 20% methanol. Membranes were blocked for 1 h with 5% non-fat dry milk and were incubated with rabbit polyclonal antibodies to TCTP (dilution 1:2000) , AKT (dilution 1:2000) and p- AKT(#4060, cell signaling, dilution 1:1000) at 4℃ overnight. After washing, membranes were incubated for 1 h at room temperature with horseradish peroxidase-conjugated secondary anti-rabbit IgG (dilution 1:5000). As an internal standard to confirm the equal loading of the proteins, β-actin (ab6276, abcam, dilution 1:5000) was loaded into the gels. Images were captured by VersaDoc 4000MP system (Bio-Rad, Hercules, CA). ImageJ was used to quantify the optic density of the Western blot bands. The optic densities of the protein bands for TCTP and β-actin were measured, and

the quantification reflects the relative amounts as a ratio of the TCTP, AKT and p- AKT protein band relative to the β-actin protein band.

Measurement of SCFAs in rumen fluid

The rumen fluid were collected by straining rumen content through four layers of cheesechoth, and frozen in liquid nitrigon. The rumen fluid were sent to Hangzhou Halo Medical Technology Co., Ltd. for SCFAs measurement.

Statistical analysis

Unless stated otherwise, data were represented as means±sem. Group comparisons were analyzed by using independent samples t-test and one-way ANOVA for parametric data using with SPSS 18.0 software (SPSS Inc., Chicago, IL, USA). p <0.05 was considered statistically significant.

Results

TCTP is expressed in rumen epithelium cell throughout postnatal period

To probe the potential roles of TCTP in rumen development, we first examined whether TCTP is presented in rumen epithelia. We detected mRNA transcripts for TCTP in rumen epithelium from birth to day 60. Among all the time points, we found the expression level of TCTP mRNA transcripts was the highest in the rumen epithelium of day 0, while it decreased to a relatively low level on day 15. Then the TCTP mRNA expression in rumen epithelium gradually increased along with the age of sheep to day 45 and it decreased to a relatively low level at the age of day 60 (Fig. 1b).

We then detected the protein expression of TCTP in rumen epithelia. Different from its mRNA expression, we found TCTP protein expression remained in a relatively low level from day 0 to day 15 of postnatal life. TCTP protein expression then significantly increased at day 30 of age (p<0.05) and gradually decreased until day 60 (Fig. 1c and d).

To better evaluate the expression of TCTP in rumen epithelia, we also determined the localization of TCTP on rumen epithelium before weaning. We found TCTP expressed in stratum basale cells at the age of day 0. On day 15 of life, TCTP was only expressed in stratum basale cell and stratum spinosum cell, while we found TCTP expressed in stratum basale, stratum spinosum and stratum granulosum after day 30 of postnatal life (Fig. 1a).

At last we evaluated the intensity of TCTP staining in rumen epithelium. We found the a few staining of TCTP in stratum basale on day 0 of postnatal life. The staining then changed from faint to strong from day 15 to day 45 of age and decreased to moderate on day 60. On day 30 and day 45 of age, we found relative stronger staining of TCTP on the top of the papillae comparing with the staining in the middle or the bottom of papillae. The staining of TCTP followed the similar pattern in stratum spinosum and stratum granulosum. We found no staining of TCTP in stratum corneum (Table 2).

Table 2 Intensity of immunohistochemical staining of TCTP in rumen epithelia

	D0	D15	D30	D45	D60
stratum basale	+/-	+/-	++	++	+
stratum spinosum	-	+	++	++	+
stratum granulosum	-	+/-	+++	+++	+
stratum corneum	-	-	-	-	-

Note that: +/- means a few staining; + means faint staining; ++ means moderate staining; +++ means strong staining;

The histological and morphological changes of ruminal papillae during postnatal stage

To determine the function of TCTP during sheep rumen papillae development, we then evaluated its histological and morphological changes from birth to 60 days old. The body weight and rumen weight increased following the age of sheep. However, the papillae length decreased from 621.8±9.2μm to 538.1±17.3μm at first 15 days of postnatal life. Thereafter, the papilla undergo a period of growth from 538.1±17.3μm to 2211.1±56.6μm until 60 days of age (Table 3). The proportion of rumen weight to the whole stomach decreased in the first 15 days of life and gradually increased to 64.40±1.33% afterward (Table 4). The growth rate of rumen papillae was -5.58μm per day from day 0 to day 15 of age. Then it became 50.50μm per day at the stage of day 15 to day 30, afterward it decreased to 31.20μm per day (Table 5).

On the first day of Hu Sheep postnatal life, the papillae were formed by a single layer of stratum basale cells that covered papillary core. The papillary core was covered by several layers of stratified squamous cells. Outside the stratified squamous cells were covered by serosa. At the age of day 15, the rumen epithelia formed tongue-shaped papilla, which were constituted by stratum basale, stratum spinosum and stratum granulosum. On this stage, the papillary cores were larger than that of day 0 and all the papillae were closely connected. At 30 days of age, we found the space between papillae larger than that of day 15, and one or two layers of stratum corneum could be clearly observed in most papillae. The papillae were also covered by serosa. At 45 days of age, the stratum corneum covered all the papillae except the top of papillae, which were still covered by serosa. In spite of more layers of stratified squamous cells, we didn't found significant histological changes of ruminal papillae at 60 days of age, when comparing with day 45 (Fig. 2).

Table 3 The papillae length, bodyweight and rumen weight of Hu sheep at different ages

	age				
	Day 0	Day 15	Day 30	Day 45	Day 60
Papillae length(μm)	621.8±9.2^{b}	538.1±17.3^{a}	1295.6±27.1^{c}	1739.8±73.0^{d}	2211.1±56.6^{e}
Body weight(kg)	3.49±0.11	6.91±0.13	9.90±0.25	13.89±0.37	18.08±0.68
Rumen weight(g)	6.7±0.4	16.2±1.3	52.1±7.4	164.5±13.9	288.3±30.0

Note: data are presented as mean±sem, n=5, p<0.05 is considered significance.

Table 4. The proportion of rumen, reticulum, omasum and abomasum weight to the whole stomach %

age	Rumen	Reticulum	omasum	abomasum
0	30.99±0.92^{d}	11.57±1.13^{a}	9.10±1.23^{a}	48.34±1.10^{b}
15	24.48±0.81^{e}	8.74±1.07^{b}	6.88±0.48^{ab}	59.91±1.54^{a}
30	43.27±2.73^{c}	7.31±0.24^{b}	6.13±0.32^{b}	42.28±2.80^{b}
45	58.92±0.75^{b}	9.88±0.85^{a}	4.47±0.36^{c}	26.73±1.15^{c}
60	64.40±1.33^{a}	10.93±1.14^{a}	5.85±0.17^{b}	18.82±0.47^{d}

Note: data are presented as mean±sem, n=5, p<0.05 is considered significance.

Table. 5 The average growing rate of papillae length at different stage μm/day

	stage			
	Day 0-day 15	Day 15-day 30	Day 30-day 45	Day 45-day 60
growth rate	-5.58	50.50	29.61	31.20

TCTP is involved in stratum basale cell proliferation

We then used Ki67 as indicator of cell proliferation to determine the role of TCTP in rumen papillae growth. We found faint staining of Ki67 in the cytoplasm of stratum basale cell and stratum spinosum cell, while the strong staining that indicated cell division were only detected in stratum basale cells (Fig. 3 a). Hence, we have counted the number of Ki67 positive cells in stratum basale. The ratio of Ki67 positive cells in stratum basale remained in a very low level from day 0 to day 15 of sheep postnatal life. It then significantly increased at day 30 of age and remained until day 45 of age (p<0.05). Finally, the ratio significantly decreased on day 60 (p<0.05) (Fig. 3 b).

AKT expression and phosphorylation during Hu Sheep postnatal life

To explore the relationship between AKT signaling pathway and TCTP, we first determined the localization of AKT in rumen epithelia tissue. We found strong staining of AKT in the stratum basale cells of day 0 sheep. The staining then turned to faint in day 15 stratum basale cell. In day 30, day 45 and day 60 sheep rumen epithelia, we found moderate staining of AKT in stratum basale cell, stratum spinosum cell and stratum granulosum cell (Fig. 4 a). Then we evaluated the AKT phosphorylation by western blot. The phosphorylation of AKT was relative low on day 0, while it significantly increased on day 15 (p<0.05). Then the AKT t phosphorylation level gradually decreased until day 45. On day 60 of age, the AKT phosphorylation level was a little bit higher than that of day 45 (Fig. 4 b and c).

The changes of SCFAs concentration in rumen fluid during Hu Sheep postnatal life

Since the SCFAs are associated rumen papillae growth, we also measured the SCFAs in the rumen fluid of Hu Sheep before weaning. We found no rumen fluid on day 0 of age, and among all the five Hu Sheep we chose to sacrifice, only one rumen contained a few rumen fluid, which was not enough for SCFAs measurement (data not shown). The concentration of acetic acid in rumen fluid was relative lower on day 30 and day 45 of age, while it significantly increased to 85.34±3.37 μmol/ml on day 60 (p<0.05). The concentration of propionic acid on day 30 was 10.48±4.28 μmol/ml, then it significantly increased to 73.55±8.39 μmol/ml on day 60 of age (p<0.05). The valeric acid concentration on day 60 also significantly rose on day 60 comparing with that of day 30. We didn't find significant change on the concentration of butyric acid, isobutyric acid and isocaleric acid. Since the ratio of acetic acid/propionic acid was considered important in rumen development, we found its ratio was 6.19±2.46 on day 30, then it significantly decreased from day 45 to day 60 (p<0.05) (Table 6).

Table 6 SCFAs concentration in rumen fluid

	age				
	Day 0	Day 15	Day 30	Day 45	Day 60
acetic acid, μmol/ml	-	-	48.83±15.24^{b}	47.60±6.97^{b}	85.34±3.37^{a}
propionic acid, μmol/ml	-	-	10.48±4.28^{c}	31.84±13.94^{b}	73.55±8.39^{a}
butyric acid, μmol/ml	-	-	8.93±3.61	10.20±1.86	10.03±2.11
isobutyric acid, μmol/ml	-	-	0.82±0.05	0.81±0.20	0.83±0.15
valeric acid, μmol/ml	-	-	1.17±0.33^{b}	2.13±0.51^{ab}	2.86±0.25^{a}
isovaleric acid, μmol/ml	-	-	1.53±0.20	1.24±0.32	1.27±0.20
acetic acid/propionic acid			6.19±2.46^{a}	2.30±0.47^{b}	1.27±0.20^{c}

Note: data are presented as mean±sem, n=5, p<0.05 is considered significance.

Discussion

Organ size is coordinated with body growth to maintain correct proportions[11]. The role of TCTP is highly conserved in growth control [12]. In this study, we have found that sheep rumen epithelia express TCTP during postnatal life. TCTP promoted the growth of sheep rumen, as it is in other systems and species [13-16]. TCTP participates in rumen papillae growth by promoting rumen epithelia basale cell division. TCTP involved cell division is associated the ratio of acetic acid/propionic acid. Our data also indicated that TCTP mRNA translation was associated with AKT phosphorylation. These results highlight an attractive role of TCTP in the development of rumen epithelium.

Promoting the growth of rumen papillae is always the key target of ruminant animal nutrition. After birth, the sheep undergo a series of dramatic gastrointestinal transformations from monogastric animal to a ruminant[17]. During weaning, the sheep must transition from a milk-based diet that is primarily digested in the abomasum and small intestine to a solid-based one[18, 19], while the rumen capacity increases from 30% to 70% of the entire stomach weight[20]. Similar to previous studies, we found the proportion of Hu Sheep rumen weight decreased until day 15 and then increased to 64.40% on day 60, while the proportion of abomasum increased at the beginning and then decreased to 18.82% on day 60. Since rumen fluid started to appear in the rumen cavity of Hu Sheep from day 15, our results indicated that it is an important stage for a Hu Sheep to transform to a ruminant.

Transcript levels by themselves are not always sufficient to predict protein levels[21]. In this study, we found TCTP mRNA expression inconsistent with protein level in rumen epithelia. Their expression patterns in rumen epithelia were totally different from birth to 45 days old, which indicated an unusual genotype-phenotype relationship of TCTP in the development of sheep rumen papillae. Previous studies have found TCTP mRNA a highly structured mRNA shielded by ribonucleoproteins, which activates dsRNA-activated protein kinase PKR [22, 23]. TCTP plays a key role in cell survival [24], while it is reported that its mRNA translation is regulated through AKT phosphorylation[25]. Hence, we suggested that, in our study, although TCTP mRNA expression level reached the highest in the rumen epithelia of day 0, TCTP protein level remained the lowest due to the low AKT phosphorylation level at this stage. From day 15 to day 30 of age, AKT phosphorylation level in rumen epithelia was the highest. Thus, higher TCTP mRNA level on day 30 resulted in a higher protein expression level, while its protein expression remained low on day 15 of age. Our data support the viewpoint that TCTP translation is regulated by AKT phosphorylation[25].

There are various of factors influencing the development of rumen papillae. The production of SCFAs is thought necessary to stimulate the development of rumen epithelium [26]. SCFAs are the primary products of rumen fermentation contributing to rumen epithelium development [27, 28]. Previous studies have found that feeding lamb with starter not only increases the concentration of SCFAs in rumen fluid, but also upregulates mRNA expression of proliferative genes and downregulates apoptosis genes[29]. Supplementing with sodium butyrate in milk replacer is associated with increased length and width of papillae[28, 30]. Previous studies have also found acetate:propionate ratio associated with the growth of

papillae[31, 32]. Consistent with these studies, we also found ratio of acetate to propionate associated with rumen papillae growing speed, which is associated TCTP expression. Our data indicated that TCTP related rumen papillae development is associated with acetate:propionate ratio.

Conclusion

TCTP participates in rumen papillae growth by promoting rumen stratum basale cell proliferation. We suggest that the translation of TCTP mRNA is regulated by AKT t phosphorylation. Our data also indicated that TCTP related rumen papillae development is associated with acetate:propionate ratio.

Abbreviations

TCTP: translationally controlled tumor protein; SCFA: short chain fatty acid.

Declarations

Acknowledgments

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Authors' contributions

YJ initiated and directed research; KZ designed and performed experiments, analyzed data and wrote the manuscript; JW and LG were involved in sample collection, analysis and manuscript edition; YY, PL, YC, XH and CM were involved in the animal experimentation and sample collection. All authors read and approved the final manuscript.

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Availability of data and materials

The raw data for the current study are available from the corresponding author on reasonable request.

Ethics approval

All procedures were approved by the Committee for the Care and Use of Animals for Experimental and other Scientific Purposes of the Zhejiang Academy of Agricultural Sciences (Hangzhou, Zhejiang, China).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Feng, X., et al., *Genome-wide differential expression profiling of mRNAs and lncRNAs associated with prolificacy in Hu sheep.* Biosci Rep, 2018. 38(2).
2. Baldwin, R.L.t. and E.E. Connor, *Rumen Function and Development.* Vet Clin North Am Food Anim Pract, 2017. 33(3): p. 427-439.
3. Heinrichs, A.J., et al., *A 100-Year Review: A century of dairy heifer research.* J Dairy Sci, 2017. 100(12): p. 10173-10188.
4. Lesmeister, K.E., P.R. Tozer, and A.J. Heinrichs, *Development and analysis of a rumen tissue sampling procedure.* J Dairy Sci, 2004. 87(5): p. 1336-44.
5. Nishihara, K., et al., *Comparative transcriptome analysis of rumen papillae in suckling and weaned Japanese Black calves using RNA sequencing.* J Anim Sci, 2018. 96(6): p. 2226-2237.
6. Li, S. and F. Ge, *Current Understanding of the TCTP Interactome.* Results Probl Cell Differ, 2017. 64: p. 127-136.
7. Gouveia Roque, C. and C.E. Holt, *Growth Cone Tctp Is Dynamically Regulated by Guidance Cues.* Front Mol Neurosci, 2018. 11: p. 399.
8. Kubiak, J.Z. and M. Kloc, *Elusive Role of TCTP Protein and mRNA in Cell Cycle and Cytoskeleton Regulation.* Results Probl Cell Differ, 2017. 64: p. 217-225.
9. Le, T.P., et al., *14-3-3 proteins regulate Tctp-Rheb interaction for organ growth in Drosophila.* Nat Commun, 2016. 7: p. 11501.
10. Betsch, L., et al., *TCTP and CSN4 control cell cycle progression and development by regulating CULLIN1 neddylation in plants and animals.* PLoS Genet, 2019. 15(1): p. e1007899.
11. Mirth, C.K. and A.W. Shingleton, *Integrating body and organ size in Drosophila: recent advances and outstanding problems.* Front Endocrinol (Lausanne), 2012. 3: p. 49.
12. Brioudes, F., et al., *Translationally controlled tumor protein is a conserved mitotic growth integrator in animals and plants.* Proc Natl Acad Sci U S A, 2010. 107(37): p. 16384-9.
13. Hsu, Y.C., et al., *Drosophila TCTP is essential for growth and proliferation through regulation of dRheb GTPase.* Nature, 2007. 445(7129): p. 785-8.
14. Jeon, H.J., et al., *TCTP regulates spindle microtubule dynamics by stabilizing polar microtubules during mouse oocyte meiosis.* Biochim Biophys Acta, 2016. 1863(4): p. 630-7.

15. Chen, S.H., et al., *A knockout mouse approach reveals that TCTP functions as an essential factor for cell proliferation and survival in a tissue- or cell type-specific manner.* Mol Biol Cell, 2007. 18(7): p. 2525-32.
16. Liu, Z.L., et al., *CRISPR disruption of TCTP gene impaired normal development in the silkworm Bombyx mori.* Insect Sci, 2019. 26(6): p. 973-982.
17. Meale, S.J., et al., *From pre- to postweaning: Transformation of the young calf's gastrointestinal tract.* J Dairy Sci, 2017. 100(7): p. 5984-5995.
18. Guilloteau, P., R. Zabielski, and J.W. Blum, *Gastrointestinal tract and digestion in the young ruminant: ontogenesis, adaptations, consequences and manipulations.* J Physiol Pharmacol, 2009. 60 Suppl 3: p. 37-46.
19. Steele, M.A., et al., *Development and physiology of the rumen and the lower gut: Targets for improving gut health.* J Dairy Sci, 2016. 99(6): p. 4955-4966.
20. Diao, Q., R. Zhang, and T. Fu, *Review of Strategies to Promote Rumen Development in Calves.* Animals (Basel), 2019. 9(8).
21. Liu, Y., A. Beyer, and R. Aebersold, *On the Dependency of Cellular Protein Levels on mRNA Abundance.* Cell, 2016. 165(3): p. 535-50.
22. Amson, R., et al., *TPT1/ TCTP-regulated pathways in phenotypic reprogramming.* Trends Cell Biol, 2013. 23(1): p. 37-46.
23. Bommer, U.A., et al., *The mRNA of the translationally controlled tumor protein P23/TCTP is a highly structured RNA, which activates the dsRNA-dependent protein kinase PKR.* RNA, 2002. 8(4): p. 478-96.
24. Kim, M., J. Jung, and K. Lee, *Roles of ERK, PI3 kinase, and PLC-gamma pathways induced by overexpression of translationally controlled tumor protein in HeLa cells.* Arch Biochem Biophys, 2009. 485(1): p. 82-7.
25. Bommer, U.A., et al., *Growth-factor dependent expression of the translationally controlled tumour protein TCTP is regulated through the PI3-K/Akt/mTORC1 signalling pathway.* Cell Signal, 2015. 27(8): p. 1557-68.
26. Suarez, B.J., et al., *Effect of roughage source and roughage to concentrate ratio on animal performance and rumen development in veal calves.* J Dairy Sci, 2007. 90(5): p. 2390-403.
27. Mentschel, J., et al., *Butyric acid stimulates rumen mucosa development in the calf mainly by a reduction of apoptosis.* Arch Tierernahr, 2001. 55(2): p. 85-102.
28. Gorka, P., et al., *Effect of sodium butyrate supplementation in milk replacer and starter diet on rumen development in calves.* J Physiol Pharmacol, 2009. 60 Suppl 3: p. 47-53.
29. Sun, D.M., et al., *Effect of starter diet supplementation on rumen epithelial morphology and expression of genes involved in cell proliferation and metabolism in pre-weaned lambs.* Animal, 2018. 12(11): p. 2274-2283.

30. Gorka, P., et al., *Effect of method of delivery of sodium butyrate on rumen development in newborn calves.* J Dairy Sci, 2011. 94(11): p. 5578-88.
31. Zitnan, R., et al., *Influence of dietary concentrate to forage ratio on the development of rumen mucosa in calves.* Arch Tierernahr, 1998. 51(4): p. 279-91.
32. Scharen, M., et al., *The effects of a ration change from a total mixed ration to pasture on rumen fermentation, volatile fatty acid absorption characteristics, and morphology of dairy cows.* J Dairy Sci, 2016. 99(5): p. 3549-3565.

Figures

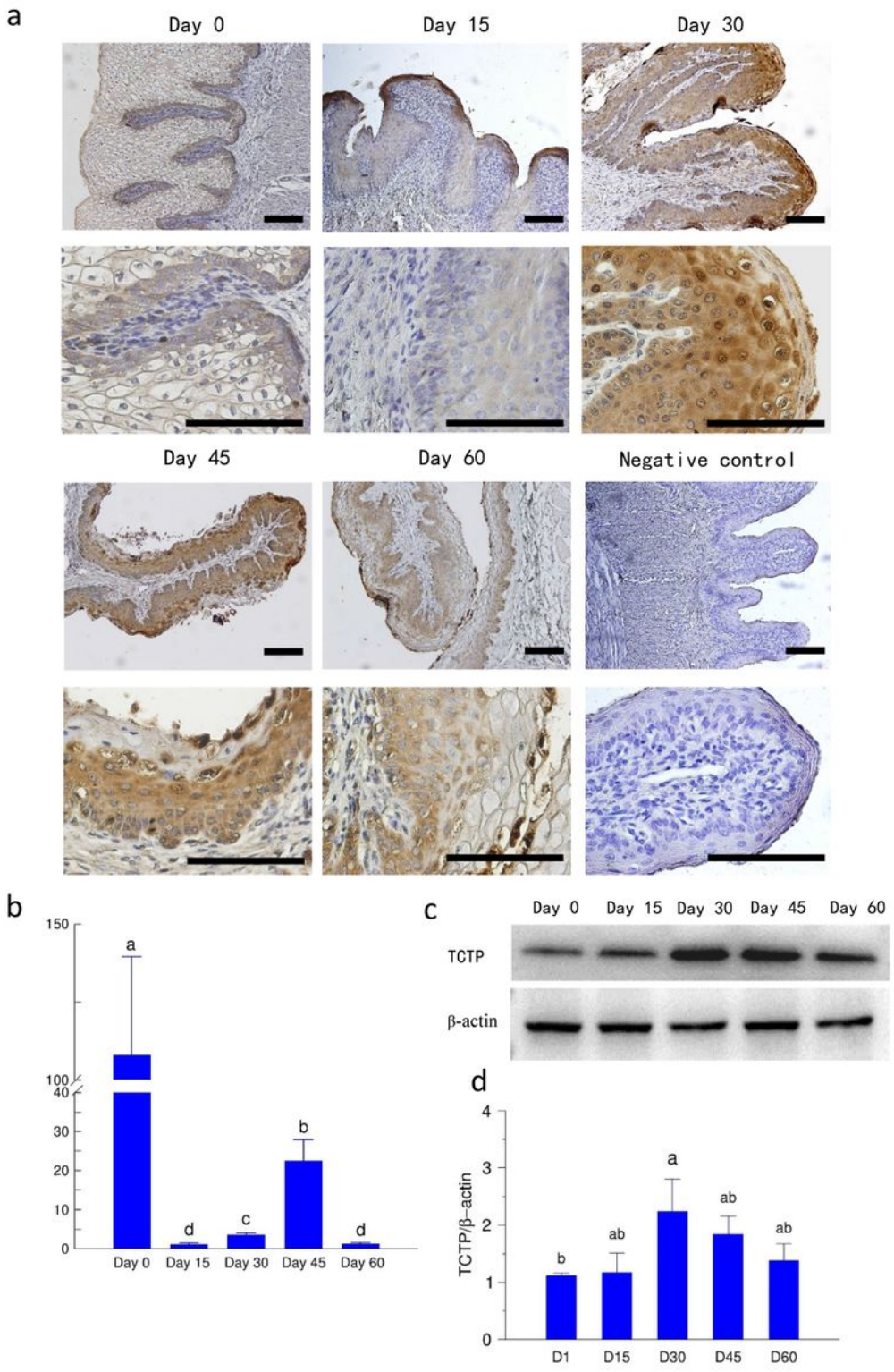

Figure 1

The expression of TCTP in rumen epithelium before weaning. (a) Immunolocalization of TCTP in sheep rumen epithelium of different stages. mRNA expression (b) and protein expression (c and d) of TCTP in rumen epithelium of different stages. Bar is 50μm. $p<0.05$.

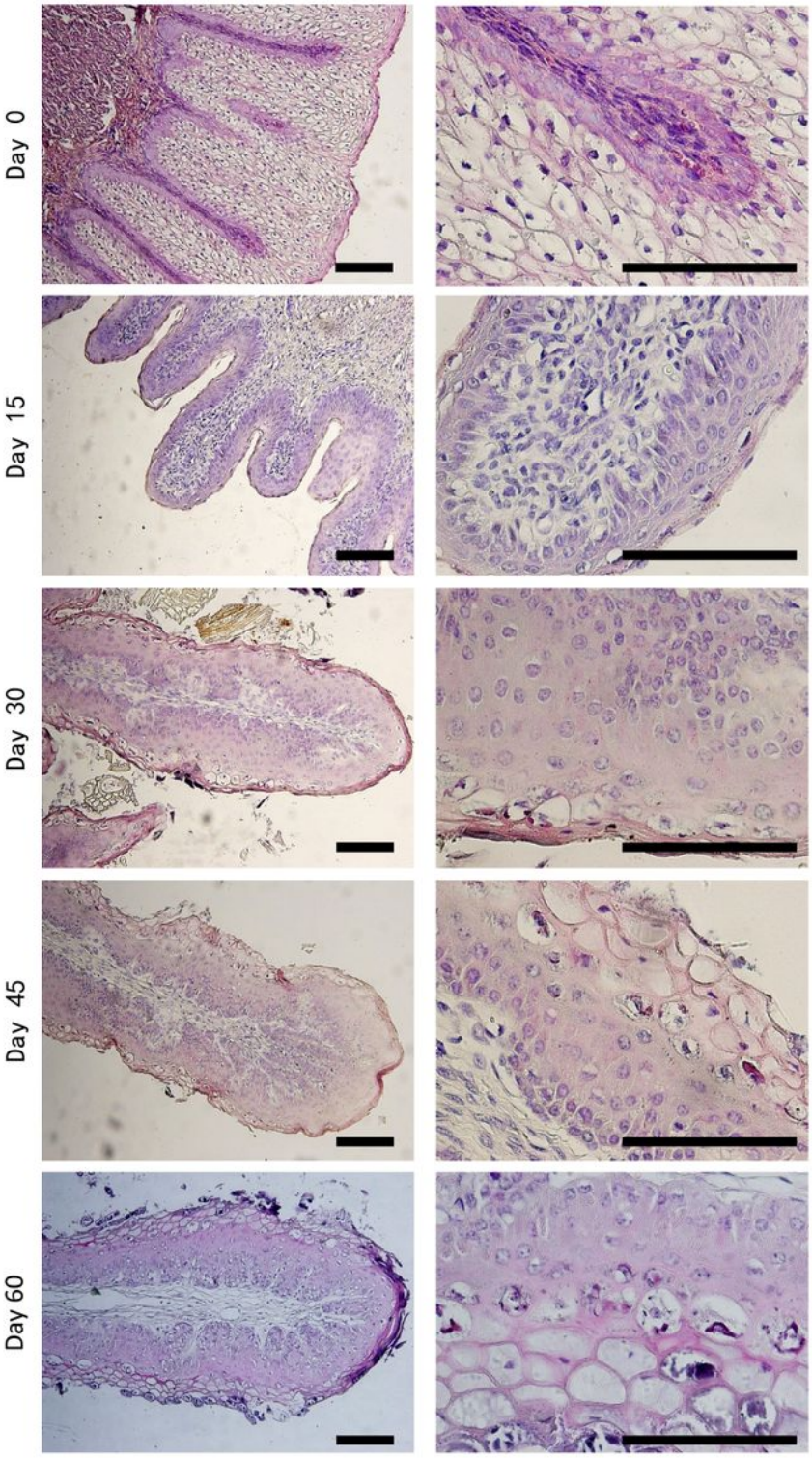

Figure 2

H&E staining of Hu Sheep rumen epithelium before weaning. Bar is 50μm.

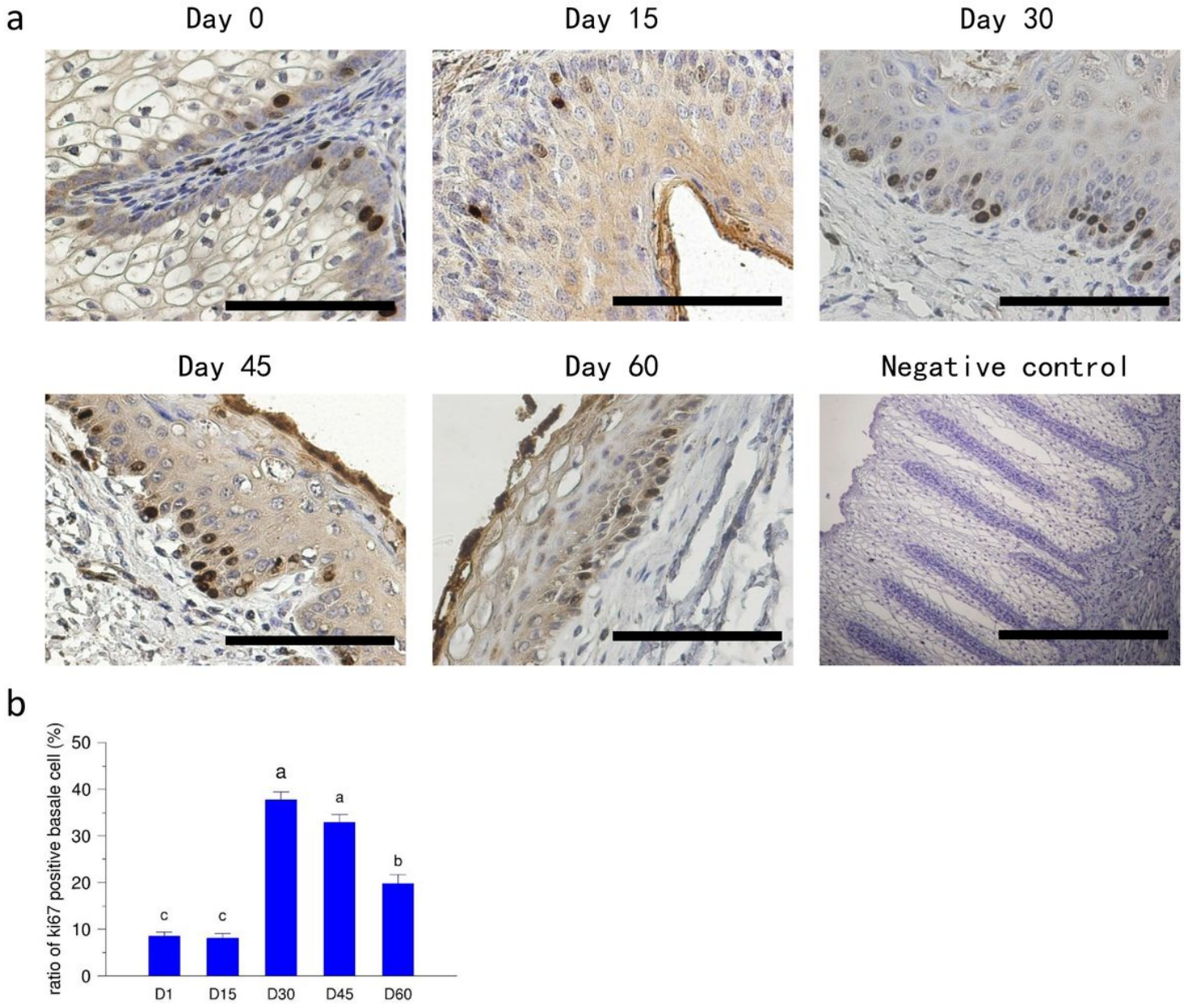

Figure 3

The ratio of Ki67 positive cells in rumen epithelium before weaning. (a) Immunostaining of Ki67 positive cells in sheep rumen epithelium of different ages. (b) The ratio of Ki67 positive cell at different ages. Bar is 50μm. $p<0.05$.

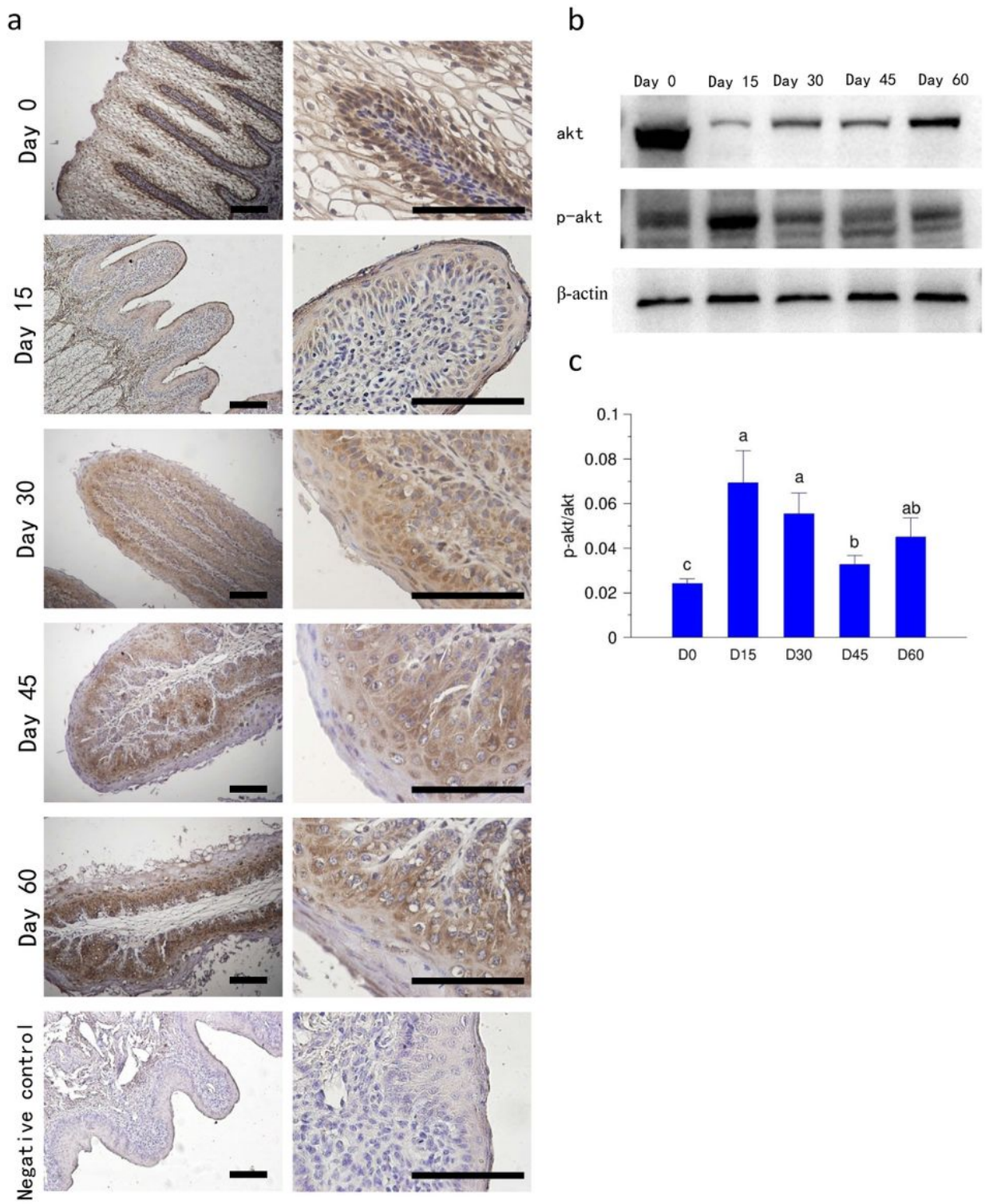

Figure 4

AKT expression and its phosphorylation in sheep rumen epithelium before weaning. (a) Immunolocalization of AKT in sheep rumen epithelium of different stages. (b) Western blot shows the expression of AKT and p-AKT in sheep rumen epithelium of different ages. (c) The phosphorylation level of AKT in rumen epithelium at different stages.