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Article

Synergistic Impact of Organic Acids on Rabbits' Digestive Physiological Processes and Health

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Simple Summary: Rabbits' productivity depends on, primarily, digestive processes and general state of health. Specific additives can positively affect rabbits' health by ensuring further production quality. Information about organic acids mixtures effect on rabbits' growth performance and physiological status is limited. So, this study aimed to investigate the effects of dietary organic acids inclusion on rabbits' growth performance, physiological indicators, and gut morphology. Forty weaned rabbits aged 28-d randomly assigned into 2 groups and fed with standard compound diet (SCD) and SCD + 0.2% organic acids mixture (butyric, propionic, sorbic) (OAM). Results indicate that OAM inclusion into feed by its synergistic action positively affects most digestive physiological processes, which reflects in general Californian rabbits' health state and feed digestibility.

Abstract: Understanding the implications of organic acids in animal diets on physiological markers is critical for developing viable strategies for improving overall health and performance. The aim of this study was to evaluate the influence of dietary organic acids inclusion on rabbits' growth performance and physiological status by determining blood and intestine development indicators. Forty weaned rabbits aged 28-d randomly assigned into 2 groups and fed with standard compound diet (SCD) and SCD + 0.2% organic acids mixture (butyric, propionic, sorbic; OAM). Growth performance was evaluated between 28-77 d of age, physiological status at 77 d of age. OAM's rabbits final body weight, ADG, DFI, growth rate indicators were higher, but FCR lower compared to SCD ($P < 0.05$). OAM increased total protein and albumin levels in blood plasma; reduced pH, increased DM, achieved greater viscosity in *caecum's* content ($P < 0.05$). Compared to SCD better gut development observed in OAM, as total intestinal weight was significantly higher ($P < 0.05$). Acetic and propionic acids concentrations were increased, but butyric reduced in OAM *caecum's* content ($P < 0.05$). OAM had significant effect on the most *caecum* histomorphometric parameters: increased *villus* height, crypt depth and their ratio ($P < 0.05$). According to our results OAM can improve rabbits' growth performance, nutrient intake, and feed digestibility by significantly improving physiological status.

Keywords: organic acids; growth performance; intestine; short-chain fatty acids; histomorphology; rabbits

1. Introduction

High-quality and safe products guarantee humans' health and well-being by creating trust between the production and consumption chains. Everyday animals' breeders chase modern trends in animal production by looking for innovative solutions to improve production quality without losing sight of animal welfare aspects. Whereas healthy and well-bred animals are the equivalent of high-quality production. For example, rabbit's meat has high nutritional value properties and many health benefits to consumers. Such meat meets the requirements of the highest quality products and is of great interest to consumers who promote a healthier lifestyle [1]. Increasing rabbit's meat consumption and demand for quality produce encourage to use not only standard feeds in rabbits' diets, but also feeds supplemented with various dietary additives. Specific additives can positively affect rabbits' health by ensuring further production quality. In general, animals' productivity depends on, primarily, digestive processes and general state of health. Therefore, the effect of livestock diets on digestive processes and meeting their body needs to be considered.

Organic acids are products of microbial metabolism, which can be supplemented into rabbits' feed. All organic acids are naturally occurring in plant or animal substrates as nutrient components generated by the normal biochemical metabolic processes [2]. Organic acids act by reducing the intestinal pH in the gastrointestinal tract and stimulates the secretion of digestive enzymes, increases the activity of proteolytic enzymes, enhances gut growth and morphological healing when they are damaged. Supposedly, that acidifiers have an antimicrobial effect by supporting the microbial balance in the intestines inhibiting the proliferation of pathogenic bacteria [3]. For example, in Europe organic acids are often added to monogastric animals' feed as acidifiers or preservatives to replace antibiotics as growth promoters seeking to pathogen prevention [4]. Organic acids are commonly considered safe, healthy and have been allowed to be used as feed additives in animals' diets by most member states of the European Union [5].

Most animal studies on alternative antibiotic applications are carried out in pigs and poultry. However, the application of such findings to rabbits' digestion physiology is not always possible because of differences between digestive systems [6]. Only few studies with organic acids usage in rabbit breeding are carried out and these results by no means are consistent [7]. Scientific literature provides sufficient data on rabbits' productivity and physiological indicators, when organic acids are used separately. And a mixture of organic acids appeared to be more effective than organic acids used separately [8]. However, information about organic acids mixtures effect on rabbits' growth performance and physiological status is limited. Thus, our study aimed to investigate the effects of dietary organic acids inclusion on rabbits' growth performance, physiological indicators, and gut morphology.

2. Materials and Methods

2.1. Experimental design

The feeding trial was carried out with forty weaned Californian rabbits, aged 28 d and with 483 ± 20 g body weight. All 40 rabbits were randomly assigned into 2 treatments (n=20/group). To guarantee optimal health conditions and maximum performance of rabbits, animals were farmed indoors in individual cages. The animals were housed in wire cages with grid floor (35×35×60 cm; 1 rabbit per cage) and had free access to individual vessel for clean drinking water and feed. Building heating system in standard conditions-maintained $19 \pm 2^\circ\text{C}$. Housing standards were in accordance with the EU Directive 98/58/EC 1998 regarding the minimum standards for the protection of animals bred or kept for farming purposes.

Rabbits fed twice a day with a standard compound diet (SCD) and with standard compound diet supplemented with 0.2% organic acids mixture (OAM). Organic acids mixture consisted with butyric, propionic and sorbic acids. Standard compound diet was formulated and analysed to cover the nutrient requirements of growing rabbits including vitamins and minerals as recommended in National Research Council (NRC) [9] (Table 1). Throughout the study both groups received the diets twice a day with *ad libitum* access to the feed, which form was pellets.

Table 1. Chemical composition¹ of the commercial diet.

Indicator ¹	Value (%)
Crude protein	16.40
Crude fibre	16.39
Starch	9.56
Sugar	4.38
Total lysine	0.65
Methionine + cysteine	0.65
Tryptophan	0.20
Linolenic acid	1.04
Threonine	0.61
Total methionine	0.39
Available phosphorus	0.37
Calcium	1.29
Phosphorus	0.59
Sodium	0.25
Chlorine	0.54

Note: ¹ Composition: barley, lucerne, wheat bran, sunflower expeller, soybean meal, premix ². ² 1 kg of premix contains: vitamin A 10.08 TV; vitamin D₃ 1.14 TV; vitamin E 50.30 mg; vitamin K₃ 0.99 mg; vitamin B₁ 3.71 mg; vitamin B₂ 2.80 mg; vitamin B₅ 9.80 mg; vitamin B₁₂ 0.01 mg; nicotinic acid 20.40 mg; folic acid 0.22 mg; choline chloride 170.00 mg; magnesium 76.28 mg; iron 317.00 mg; zinc 110.89 mg; copper 19.16 mg; cobalt 0.29 mg; iodine 0.67 mg; selenium 0.31 mg.

2.2. Experimental protocol and sample collection

Rabbits were weighed individually at the beginning (28 d) and at the end of the trial (77 d). The average daily weight gain (ADG), daily feed intake (DFI), feed conversion ratio (FCR) and growth rate were calculated for each experimental unit.

At the end of the trial 20 rabbits (n=10/group) were randomly selected, weighed, starved overnight, and then euthanized in accordance with normal farming practice. They were bled within two minutes after physical stunning. Blood samples were collected into dry, clean tubes and allowed to clot for 30 minutes, blood plasma was separated by centrifugation at 3000 rpm for 10 minutes. Post-mortem from each rabbit digestive tract was removed.

2.3. Physiological properties assay

The following parameters has been established in blood plasma based on methodology by Tietz [10]. Parameters determined: total protein, albumin, globulin, triglycerides, cholesterol, AST and ALT levels. Rabbits' blood plasma was analysed with blood analyser COBAS-Integra 400/700/800 (Roche Diagnostics, USA).

pH of *duodenum* and *caecum* contents was carried out using pH-meter "Inolab 730" (WTW, Germany).

Dry matter (DM) of *duodenum* and *caecum* was measured by drying their content at 105°C for 3 hours and calculating the difference between the dried and non-dried samples of the intestinal sections [11].

The viscosity of *caecum's* content was measured at a shear rate of 30 s^{-1} using a Brookfield Digital DV-II cone/plate viscometer (Brookfield Engineering Laboratories Inc. Stoughton, MA, USA). Approximately 2 g (wet weight) of the fresh digesta were immediately placed in a microcentrifuge tube and centrifuged at $12,700 \times g$ for 5 min for *caecum's* digesta viscosity analysis. The supernatant was withdrawn and stored on ice until viscosity ($\text{mPa}\cdot\text{s} = \text{cP} = 1 \times 100 \text{ dyne s cm}^{-2}$) was determined using viscometer at 40°C .

Digestive tract was removed and weighed post-mortem. Each intestinal segment was weighed as well. The length of every intestinal segment measured with flexible tape Hoechstmass (Hoechstmass, Germany) on a glass surface. The intestinal walls were washed with physiological solution, dried up with filter paper and weighed again [12].

The profile of the short-chain fatty acid (SCFA) in *caecum's* content was determined by using a gas chromatography system Shimadzu GC – 2010 (Shimadzu Corp., Japan) with a glass tube 2.5 mm x 2.6 mm, filled in with 10% of SP -1200/ 1% HPO on 80/100 Chromosorb W AW, tube temperature 110°C , detector's FID temperature 108°C , injector's temperature 195°C . The value of the SCFA accumulation calculated as a concentration of separate SCFA in a digestive content [13].

The quantities of ammonia nitrogen (N-NH_3) in *caecum's* content were determined with gas chromatography system (Shimadzu GC-14A; Shimadzu Ko, Tokyo, Japan) according to Foss-Tecator method ASN 3302.

Post-mortem histopathological mucosal histomorphometry was conducted in 3 parts of *caecum* (PRO; MID; DISTAL). Samples were fixed with 10% neutral formalin solution. Using standard procedures for histologic evaluation the tissues were embedded into paraffin, cut with rotary microtome by $4 \mu\text{m}$ thick tissue sections, which were painted with hematoxylin and eosin. Prepared histological samples were examined by using Olympus BX63 microscope (Olympus Corp., Tokyo, Japan), Olympus DP72 video camera (Olympus Corp., Tokyo, Japan) and the computer Image Pro Plus program system for Windows, version 7.0 (Media Cybernetics, Inc., Bethesda, MD, USA, 2009). Both groups of rabbits' *caecum's villus* height and crypt depth were morphometrically and microscopically measured, V/C ratio calculated.

2.4. Statistical analysis

All results are presented as mean \pm standard error of mean (SEM). Data analysis was performed by SPSS for Windows, version 25.0. Parametric Independent T-test, which compares the means of two independent groups to determine whether there is statistical evidence that the associated population means are significantly different, was conducted to detect differences among groups. A calculated P value of less than or equal to 0.05 ($P < 0.05$) was considered statistically significant.

3. Results and discussion

3.1. Growth performance

In Europe acidifiers or preservatives such as organic acids are often used in monogastric animals' feed production to replace antibiotics and to ensure protection from pathogens [4]. When evaluating animals' productivity traits, feed supplementation with organic acids supports better feed conversion ratio (FCR), promotes growth, improves minerals absorption and body weight gain [14]. Influence of dietary organic acids mixture inclusion on rabbits' growth performance is shown in Table 2. This kind inclusion improved rabbits' weight at 77 d of age: compared to SCD, OAM group's rabbits were 106.60 g heavier ($P < 0.05$). Similar study with organic acids mixture (dosage 5 g to 1 kg feed) was performed by Cardinali et al. [15]. In his study with 28-day old, weaned rabbits' positive effect was obtained on weight gain in the second phase of fattening. Our research results after counting average daily gain (ADG) and daily feed intake (DFI) showed that feed supplemented with OAM significantly increased mentioned productivity indicators ($P < 0.05$). Other scientists' research results show that ADG can be increased even when using other kind organic acids separately or together as mixtures. For example, ADG improvements observed especially when using fumaric acid [16], lactic and formic acids mixtures [17], butyric acid [18,19]. However, not in all scientists' studies

such results were observed [20,21]. The positive results of growth performance are usually associated with intestinal morphological changes and improvements in feed digestibility [19,22]. On the contrary to ADG results, feed conversion ratio (FCR) of OAM group was slightly lower compared to SCD ($P < 0.05$). Similar to our research was performed by Zeweil et al. [23]. However, on the contrary to ours, his study showed a significantly better FCR while using dosage of 0.05% propionic acid in feed. Nevertheless, the growth rate of our study's fattening rabbits was slightly higher in group supplemented with organic acids mixture, comparing to standard compound diet ($P < 0.05$). These beneficial results on growth performance appeared to be because used organic acids mixture, which lowered gastrointestinal tract's pH and facilitated nutrient retention. This observed positive trend could be related to more favourable microbiota in the digestive tract and improved digestion of the feed. The specific antimicrobial activity of OAM, which could have contributed to the reduction of intestinal bacteria due to low bacterial competition with the host for available nutrients and a decrease in the levels of harmful bacterial metabolites linked to reduced bacterial fermentation leading to improved feed digestibility.

Table 2. Influence of dietary organic acids inclusion on rabbits' growth performance between 28 and 77 d of age (n=20 rabbits/group).

Indicator ¹	SCD ²	OAM ³	P value ⁴
Body weight 28 d (g)	483.33 ± 6.48	482.93 ± 3.08	0.846
Body weight 77 d (g)	1296.76 ± 3.46	1403.36 ± 6.65	0.000*
ADG (g)	16.65 ± 0.66	18.80 ± 0.62	0.000*
DFI (g)	78.25 ± 0.98	82.25 ± 1.34	0.000*
FCR (kg/kg)	4.72 ± 0.07	4.39 ± 0.07	0.000*
Growth rate	1.68 ± 0.04	1.90 ± 0.03	0.000*

Note: ¹ADG, average daily weight gain; DFI, daily feed intake; FCR, feed conversion ratio. ²SCD, standard compound diet. ³OAM, SCD + 0.2% organic acids mixture. ⁴Means between groups significantly differ with * superscript ($P < 0.05$).

3.2. Physiological properties

For an objective to investigate the state of rabbits' health, determination of blood composition was carried out during our study. It is important parameters of animal's body physiological state, which associated with proper functioning of vital organs and, ultimately, with animal's production traits. Table 3 shows the influence of dietary organic acids mixture inclusion on rabbits' blood parameters at 77 d of age. However, after evaluating blood plasma results no significant effects of organic acids mixture were observed when comparing globulin, triglycerides, cholesterol, AST, and ALT contents between groups ($P > 0.05$). Our results are in line with Mohamed et al. [24] who reported that supplementation of different doses of another organic acid (humic) has no effect on AST and ALT activities in rabbits' blood plasma. However, these results means that liver functions were normal under the treatment of OAM. Nevertheless, organic acids mixture inclusion increased total protein and albumin amounts in blood plasma ($P < 0.05$). The significant total protein increase in OAM rabbits' blood plasma could be due to the achieved significant increase in albumin. It indicates that supplemental organic acids mixture might improve protein synthesis in rabbit's liver and digestibility of crude fibre and organic matter. However, obtained results during our study showed that OAM treatment did not have a significant effect on the most determined blood parameters, but all observed values were within the physiological limits for rabbits [25-27]. Our present results conduct with the findings of Dorra et al. [20] and Gorlov et al. [1], who reported that blood parameters of rabbits were not affected by dietary organic acids inclusion. Research of Sherif [28] found that dietary organic acids mixture at 1.0 g/kg in feed do not affect blood plasma parameters (total protein, albumin, globulin, triglycerides, cholesterol, AST, and ALT) of New Zealand white rabbits either.

Table 3. Influence of dietary organic acids inclusion on rabbits' blood plasma parameters at 77 d of age (n=10 rabbits/group).

Indicator ¹	SCD ²	OAM ³	P value ⁴
Total protein (g/dL)	5.72 ± 0.04	5.89 ± 0.04	0.016*
Albumin (g/dL)	3.37 ± 0.08	3.59 ± 0.06	0.036*
Globulin (g/dL)	2.33 ± 0.12	2.41 ± 0.09	0.612
Triglycerides (mg/dL)	65.50 ± 1.04	64.34 ± 1.02	0.450
Cholesterol (mg/dL)	73.46 ± 1.20	75.64 ± 1.68	0.323
AST (U/L)	62.02 ± 0.87	65.74 ± 1.40	0.054
ALT (U/L)	53.80 ± 1.79	55.56 ± 2.10	0.541

Note: ¹AST, aspartate aminotransferase; ALT, alanine aminotransferase. ²SCD, standard compound diet. ³OAM, SCD + 0.2% organic acids mixture. ⁴ Means between groups significantly differ with * superscript ($P < 0.05$).

Positive results of animal's growth are usually associated with intestinal morphological changes and improvements in feed digestibility [29], reduced intestinal pH and normal intestinal microflora [23]. Organic acids mixture inclusion into rabbits' diet affected most of intestine traits at 77 d of age (Table 4). Excluding this kind of inclusion did not have any significant effects on pH and dry matter content in *duodenum* and to whole intestine length ($P > 0.05$). Organic acids act by reducing the intestinal pH in the gastrointestinal tract and stimulates the secretion of digestive enzymes, increase the activity of proteolytic enzymes, enhance gut growth and morphological healing in lesions. It is believed that acidifiers have an antimicrobial effect, they support the microbial balance in the intestines inhibiting the proliferation of pathogenic bacteria's [4]. In our study, organic acids mixture significantly reduced pH and increased dry matter (DM) in *caecum's* content ($P < 0.05$). Significantly greater dry matter in OAM groups rabbits' *caecum's* content coincides with Romero et al. [17] results, where higher quantity of dry matter content in *caecum* was established while using feed supplemented with formic and citric acids mixture. More liquidly *caecum's* content may be associated with animal morbidity [30], while our results inversely indicate increase of dry matter content in *caecum*, which means less morbidity. Increased quantity of dry matter content in *caecum* can be caused by feed, since fibre is good barrier to water and its mixture is pushed further into the large intestine, and then a large part of it is removed with solid faeces. *Caecum's* content viscosity levels could be used as an indicator of digestibility and nutrient absorption as well. After viscosity determination in our study, OAM samples of *caecum's* content had much greater viscosity compared to SCD group samples ($P < 0.05$). Increased viscosity can affect secretion of endogenous enzymes and bile acids, which causes morphological changes in the intestine and nutrient digestibility [31]. There are studies, which results proves that organic acids mixtures improve nutrient digestibility [32]. Increased viscosity in our study coincides with improved growth results, however, no insufficient data on the effects of organic acids mixture on the rabbits' *caecum's* content viscosity in the scientific literature. After weighting separate intestinal parts, total weight of whole rabbit's intestine was higher in OAM group compared to SCD ($P < 0.05$). These results were in line with other studies conducted by Uddin et al. [32] and Ghazvinian et al. [33]. Increased intestinal weight indicates better gut development, which leads to better digestion of nutrients, and this is reflected in improved growth rates. To be more precisely, addition of organic acids mixture in feed may support nutrient digestibility and bioavailability. It is possible that it acts by lowering digestive systems acidity which lead to better utilization of nutrients. It appears that organic acids mixture used in our experiment affected the metabolism of nutrients and increased the size and morphology of the digestive system. In general, epithelial cells of the gastrointestinal tract could cause this weight gain.

Table 4. Influence of dietary organic acids inclusion on rabbits' intestine traits at 77 d of age (n=10 rabbits/group).

Indicator	Intestine	SCD ¹	OAM ²	P value ³
pH	<i>Duodenum</i>	6.93 ± 0.06	6.29 ± 0.13	0.223
	<i>Caecum</i>	7.13 ± 0.33	5.87 ± 0.05	0.014*

Indicator	Intestine	SCD ¹	OAM ²	P value ³
DM (%)	<i>Duodenum</i>	16.24 ± 0.33	17.07 ± 0.33	0.109
	<i>Caecum</i>	20.14 ± 0.21	24.45 ± 0.46	0.000*
Viscosity (mPA's)	<i>Caecum</i>	0.84 ± 0.05	2.18 ± 0.29	0.002*
Weight (g)	Total	270.33 ± 6.31	296.20 ± 8.19	0.037*
Length (cm)	Total	499.67 ± 9.02	504.12 ± 9.60	0.744

¹SCD, standard compound diet. ²OAM, SCD + 0.2% organic acids mixture. ³Means between groups significantly differ with * superscript ($P < 0.05$).

Influence of dietary organic acids mixture inclusion on rabbits' short-chain fatty acids profile and ammonium nitrogen content in *caecum's* content at 77 d of age is shown in Table 5. Short-chain fatty acids (SCFA) is a regular source of energy for rabbits. Both, SCFA and ammonium nitrogen (N-NH₃), are absorbed into the bloodstream from *caecum*. The concentration of N-NH₃ in *caecum's* content can be determined by several factors: H₂ pressure, reaction content, carbohydrate availability. As compared to ruminants, proteolytic activity is higher in the rabbit *caecum* [18]. Unfortunately, no significant effects of OAM treatment were noted on lactic acid content and N-NH₃ concentration in *caecum's* content ($P > 0.05$). The profile SCFA of rabbits' *caecum's* content is specific: acetate is prevailing (65-87 mmol per 100 ml⁻¹), followed by butyrate (6-28 mmol per 100 ml⁻¹) and propionates (3-11 mmol per 100 ml⁻¹). The proportion depends on fibre content in the diet. For example, when increasing dietary fibre, it is usually noticeable on butyrate reduction and the proportion of acetates increasing. This proportion is reflected in our research results: amounts of acetic and propionic acids were increased by OAM and were slightly higher compared to SCD ($P < 0.05$). On the contrary, OAM decreased butyric acid, which content was lower than in SCD ($P < 0.05$). Rabbits are special in assessing microbial activity of their body, it should therefore be noted that butyric acid is more important SFCA than propionic, which can be critical for health assessment and prevention of enteritis. Changes in SCFA profile could lead to changes of commensal intestinal microflora composition, which cannot be assessed without a microbiological analysis.

Table 5. Influence of dietary organic acids inclusion on rabbits' short-chain fatty acids (SCFA) and ammonium nitrogen (N-NH₃) levels in *caecum's* content at 77 d of age (n=10 rabbits/group).

Indicator ¹	SCD ²	OAM ³	P value ⁴
SCFA (µmol/g)			
Acetic acid	38.37 ± 5.46	53.04 ± 2.90	0.021*
Propionic acid	5.13 ± 0.72	6.73 ± 2.21	0.001*
Butyric acid	4.15 ± 1.90	1.19 ± 0.79	0.013*
Lactic acid	2.39 ± 0.90	1.83 ± 0.81	0.793
N-NH ₃ (mg/g)	1.84 ± 0.10	1.63 ± 0.12	0.208

Note: ¹SCFA, short-chain fatty acids; N-NH₃, ammonium nitrogen. ²SCD, standard compound diet. ³OAM, SCD + 0.2% organic acids mixture. ⁴Means between groups significantly differ with * superscript ($P < 0.05$).

One of the most important properties of organic acids is their influence on intestinal histomorphology. It is known that because of organic acids (particularly, fatty acids such as butyric acid), growth of the lining of the digestive tract becomes more intensive. Butyric acid is an important source of energy for intestinal epithelial cells, it stimulates the proliferation and differentiation of cells, prevents growth of cancer cells [18]. Organic acids mixture effect on rabbits' *caecum's villus* height, crypt depth and V/C ratio at 77 d of age is shown in Table 6. All histomorphometric results obtained in our study showed statistically significant differences between groups. Except, V/C ratios in DISTAL part of *caecum* did not differ between groups ($P > 0.05$). OAM group's results showed significantly better results by increasing intestinal *villus* height in all parts of *caecum* (PRO; MID; DISTAL) ($P < 0.05$). Longer intestinal *villus* indicates absorption surface increase, and thus better approachability to nutrient absorption. Also, these results are in line with growth performance improvements achieved in OAM group. Intestinal epithelial cells, appearing in crypts, migrate by *villus* surface to the summit and are squeezed into the intestinal lumen within 48-96 h [18]. In case of

shorter *villus* may result in a weaker nutrient absorption, increasing secretion in the digestive tract and worse growth performance [34]. Meanwhile, elongation of *villus* and improvement of V/C ratio directly correlates with the increasing changes of epithelial cells [35]. It is believed that the increase of *villus* height indicates their functions activity [36]. Therefore, it can be concluded that the rabbits' intestinal *villus* functions activity was improved after feed supplementation with organic acids mixture. This coincides with the studies conducted by other scientists [15,17,18,24,37,38]. Comparing our results to others we can agree and conclude that improved crypt depth has increased crypt cell production or an indicator of intensified mucus secretion [39] and V/C ratio reflects the digestive capacity of the intestine [40].

Table 6. Influence of dietary organic acids inclusion on rabbits' *caecum's* intestine *villus* height, crypt depth and V/C ratio at 77 d of age (n=10 rabbits/group).

Part of <i>caecum</i> ¹	Indicator	SCD ²	OAM ³	P value ⁴
PRO	<i>Villus</i> height (μm)	571.64 ± 15.03	658.22 ± 2.97	0.023*
	Crypt depth (μm)	234.64 ± 17.58	246.12 ± 2.43	0.005*
	V/C ratio	2.49 ± 0.19	2.68 ± 0.03	0.013*
MID	<i>Villus</i> height (μm)	425.11 ± 2.46	533.08 ± 3.16	0.000*
	Crypt depth (μm)	212.48 ± 1.74	252.32 ± 1.56	0.000*
	V/C ratio	2.00 ± 0.01	2.11 ± 0.02	0.000*
DISTAL	<i>Villus</i> height (μm)	378.08 ± 2.98	408.90 ± 2.59	0.000*
	Crypt depth (μm)	128.48 ± 1.39	138.14 ± 1.60	0.002*
	V/C ratio	2.94 ± 0.05	2.96 ± 0.02	0.779

Note: ¹PRO, proximal segment; MID, middle segment; DISTAL, distal segment. ²SCD, standard compound diet. ³OAM, SCD + 0.2% organic acids mixture. ⁴ Means between groups significantly differ with * superscript ($P < 0.05$).

4. Conclusions

Present findings demonstrate that dietary supplementation with organic acids mixture (butyric, propionic, sorbic) firstly could be a potentially used to improve rabbits' growth performance indicators during fattening period. Based on the physiological status determined in our study, organic acids mixture increases protein and albumin levels in blood plasma, helps to achieve greater viscosity and dry matter in *caecum's* content. Under organic acids mixtures treatment rabbits attains superior intestinal development and histomorphometric parameters (increased *villus* height, crypt depth, V/C ratio). These achievements indicate increased digestive surface areas, which is directly correlated with better digestion and ingenuously with better overall animals' health. Therefore, we can conclude that organic acids mixture 0.2% inclusion into feed by its synergistic action positively affects most digestive physiological processes, which reflects in general Californian rabbits' health state and feed digestibility.

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pain, suffering, distress or permanent injury to the animals equal to or more severely needle pricked. In this study, the animals were kept in standard farm conditions. Thus, ethical approval was not required.

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