

## Determination of buffering capacity of the selected feeds used in swine nutrition

### Stanovenie pufráčnej kapacity vybraných krmív používaných vo výžive ošípaných

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#### ABSTRACT

If gastric acidity is to be encouraged, it may be beneficial to eliminate some feed elements from pig starter meals since they bind more acid in the stomach than others. The cereals (wheat, maize, and barley), soybean meal, commercial mineral supplement, and two feed mixtures (one of them with the additive Zeolit) were evaluated. In this study, it was discovered that adding zeolite to the feed mixture had the effect of lowering its initial pH ( $P < 0.001$ ), buffering ( $P < 0.05$ ) and binding capacity ( $P < 0.05$ ). This study aimed to measure the buffering and acid-binding capacity of some ingredients commonly used in growing pig feeds and estimate the coefficient of correlation between crude protein, ash, and the buffering capacity of feed ingredients with significant linear correlation ( $P < 0.05$  and  $P < 0.01$ , respectively). The combined impact of the individual feed mixture components' individual buffering capacities has not been proven.

**Keywords:** buffering capacity, acid binding capacity, pig, diet

#### ABSTRAKT

Ak sa má podporiť kyslosť žalúdka, je potrebné obmedziť niektoré zložky krmiva z krmných zmesí pre ošípané, pretože viažu v žalúdku viac kyselín ako iné. V tejto práci boli hodnotené jednotlivé komponenty, ktoré sa bežne využívajú pri výrobe krmných zmesí pre prasatá. Boli použité obilniny (pšenica, kukurica a jačmeň), sójový extrahovaný šrot, komerčný minerálny doplnok a dve krmné zmesi (jedna s prídavkom Zeolitu). V tejto štúdii sa zistilo, že prídanie zeolitu do krmnej zmesi malo za následok zníženie jej počiatočného pH ( $P < 0,001$ ), pufráčnej ( $P < 0,05$ ) a väzbovej kapacity ( $P < 0,05$ ) v porovnaní s kontrolnou krmnou zmesou. Pri sledovaní závislosti medzi pufráčnou kapacitou a množstvom dusíkatých a minerálnych látok bola zaznamenaná signifikantne zvýšená pufráčna aktivita u krmných komponentov s vyšším obsahom dusíkatých a minerálnych látok ako u iných komponentov, ktoré mali nižšie hodnoty dusíkatých a minerálnych látok ( $P < 0,05$  resp.  $P < 0,01$ ). Nebol preukázaný kumulatívny efekt pufráčnych kapacít jednotlivých krmných komponentov tvoriacich krmné zmesi. Výsledky poukázali, že analyzovaná pufráčna kapacita krmných zmesí je vyššia ako vypočítaná pufráčna kapacita na základe pufráčnych kapacít jednotlivých krmných komponentov tvoriacich krmné zmesi.

**Klíúčové slová:** pufráčna kapacita, väzobná kapacita, ošípaná, krmivo

## INTRODUCTION

Swine protein digestion starts in the stomach with the aid of the pepsin enzyme, which is created by the major stomach cells and transforms from an inactive form to pepsin in an acidic environment. Because a low pH is necessary for the conversion of gastric zymogens into active enzymes, the stomach is endowed with acid-secreting (hydrochloric acid) cells that aid in maintaining digestion. Pepsinogen converts to pepsin quickly at a pH of 2.0 but slowly at a pH of 5.0 to 6.0 (Khan et al., 1999; Yen, 2000). On the other hand, the ideal pH is between 1.5 and 3.5, and activity rapidly declines above this pH (Suiryanrayna and Ramana, 2015). The higher amount of lactate in the stomach of suckling piglets led to inhibited HCl secretion (Cranwell et al., 1976; Kidder and Manners, 1978; Yen, 2000; Lawlor et al., 2005). The pH of piglet's stomachs is higher than the optimal range because the first feed is colostrum, and for pigs as a standard protein, it is considered the protein of sow's milk (Rolinec et al., 2018), which can be affected by the health status of sows (Rolinec et al., 2012). Intake of larger amounts of feed at irregular intervals may result in increased pH values (above 5), which may persist for a few days (Kidder and Manners, 1978; Lawlor et al., 2005). The feeds' high buffering/binding capacity contributes to even higher stomach pH (Prohaszka and Baron, 1980; Jasaitis et al., 1987; Bolduan et al., 1988; Suiryanrayna and Ramana, 2015). In monogastric animals, it may alter gastrointestinal pH, which may have an effect on how proteins are digested and the health of the gut flora (Parma et al., 2019). The development of HCl secretory capacity appears to be higher in weaned pigs than in sucklings (Cranwell, 1985; Cranwell and Moughan, 1989; Jensen et al., 1997), while other studies reported reduced pepsin and lipase activities in gastric mucosa after weaning (Hedemann et al., 2004). Increased gastric pH in weaned pigs leads to decreased digestion, resulting in the fermentation of feed in the caudal part of the digestive tract, which can cause diarrhea (Bolduan et al., 1988; Yen, 2000). The addition of acidifiers to feed or administration of feeds with low buffering and binding capacity to pigs has long been common practice (Prohaszka and Baron, 1980; Jasaitis et

al., 1987; Bolduan et al., 1988; Dibner and Buttin, 2002; Che et al., 2012; Nguyen et al., 2020). However, very little is known about the ability of the different components of complex pig feed to bind. Lawlor et al. (2005) described the binding and buffering capacity of the individual components commonly used in the manufacture of compound feeds for pigs. The aim of the work was to determine the binding and buffering capacity of feed components and complete feed mixtures, which we used in feeding pigs, and to express the correlation between the buffering capacity and the content of crude protein and ash content. The last evaluation was a comparison of the analyzed and calculated buffering capacity of the feed mixtures.

## MATERIALS AND METHODS

Two feed mixtures (experimental and control) as well as all individual basal components of these two feed mixtures (soybean meal, corn, barley, and wheat) were ground to a size of <2 mm and stored in glass bottles without access to air at room temperature until analysis. The experimental feed mixture additionally contained Zeolite (in an amount of 2 kg per 100 kg of feed mixture; 2%). The composition of the experimental and control feed mixture is shown in Table 1.

**Table 1.** The composition of experimental and control feed mixture

Items	Experimental	Control
Corn (%)	35	35
Wheat (%)	18	20
Barley (%)	17.74	17.74
Soybean meal (%)	24	24
Commercial mineral supplement (%)	3	3
Lysine (L-Lysine HCl) 78% (%)	0.1	0.1
Methionine (DL) (%)	0.05	0.05
Treonine (L) 98% (%)	0.11	0.11
Zeolite (%)	2	-

The procedure for determining the buffering and binding capacity, the determination of the initial pH of the feed components and feed mixtures, and the consumption of the titration solution up to pH 3 were according to Lawlor et al. (2005). pH values were measured with a Consort C830 laboratory pH meter. A 0.5 g sample of feed mixtures and individual feed components was added to 50 ml of deionized water and stirred continuously with an electric stirrer. The initial pH was recorded three minutes after the sample was added and stirring was started. Titration was performed with acid (0.1 M HCl). The acid was added until the pH stabilized at pH 3. The binding capacity was calculated as the amount of 0.1 M HCl consumption in milliequivalents (meq) required to acidify 1 kg of feed to pH 3. The buffering capacity was expressed as the ratio of the binding capacity and the total pH difference (pH 3 was subtracted from the initial pH). The average binding/buffering capacity and initial pH of the feed components and feed mixtures was calculated from six measurements. Individual feed components and their nutritional value are presented in Table 2.

Data analysis was carried out via the GraphPad Prism 5.0 (GraphPad Software, San Diego, CA, USA). The results of each variable were expressed as mean  $\pm$  standard deviations (SD). The Unpaired Tukey's test for multiple comparison of means was conducted to compare the initial pH, acid binding capacity, and buffering capacity of the experimental and control feed mixtures, where

the addition of zeolite was set as the main factor at a significance level of  $P < 0.001$  and  $P < 0.05$ . Subsequently, the analysis of the correlation between buffering capacity and crude protein was performed with a significance level set at  $P < 0.05$  and between buffering capacity and ash with a significance level set at  $P < 0.01$ . The determined buffering capacity of the feed mixtures was compared with the buffering capacity of the individual feed components (the sum of the buffering capacities of the individual feed components in a proportional representation in the feed mixture), which were represented in the feed mixture. The nutritional composition of the experimental and control feed mixtures is shown in Table 3.

**Table 3.** Nutritional composition of feed mixture of control and experimental group

Items	Experimental (n=6)	Control (n=6)
Dry matter (%)	89.83 $\pm$ 0.04	89.85 $\pm$ 0.02
Crude protein (%)	20.36 $\pm$ 0.3	21.34 $\pm$ 0.44
Crude fibre (%)	3.77 $\pm$ 0.06	3.81 $\pm$ 0.04
Ether extract (%)	2.05 $\pm$ 0.16	2.39 $\pm$ 0.09
Ash (%)	7.51 $\pm$ 0.29	5.75 $\pm$ 0.12

Values are Means  $\pm$  SD

The nutritional composition of commercial mineral supplement and Zeolite are recorded in Tables 4–5.

**Table 2.** Nutritional values of individual feeds

Items	Soybean meal (n=6)	Corn (n=6)	Barley (n=6)	Wheat (n=6)
Dry matter (%)	87.9 $\pm$ 0.6	86.3 $\pm$ 1	87.1 $\pm$ 1.3	87 $\pm$ 1.3
Crude protein (%)	51.13 $\pm$ 1.2	10.99 $\pm$ 0.8	12.7 $\pm$ 1.1	14.48 $\pm$ 1.3
Crude fibre (%)	6.7 $\pm$ 0.9	2.5 $\pm$ 0.4	5.2 $\pm$ 0.8	2.6 $\pm$ 0.4
Ether extract (%)	2 $\pm$ 0.5	4.3 $\pm$ 0.4	2 $\pm$ 0.3	1.7 $\pm$ 0.3
Ash (%)	7.28 $\pm$ 0.5	1.9 $\pm$ 0.1	3.1 $\pm$ 0.3	2.2 $\pm$ 0.2

Values are Means  $\pm$  SD

**Table 4.** Nutritional composition of commercial mineral supplement

Items	Commercial mineral supplement
Dry matter (g/kg)	980
Crude protein (g/kg)	95
Lysine (g/kg)	80
Methionine (g/kg)	13
Methionine+Cysteine (g/kg)	13
Threonine (g/kg)	15
Ca (g/kg)	210
P (g/kg)	30
Mg (g/kg)	20
Na (g/kg)	45
Mn (mg/kg)	1750
Zn (mg/kg)	3500
Fe (mg/kg)	3500
Cu (mg/kg)	4800
J (mg/kg)	55
Se (mg/kg)	16
Co (mg/kg)	40

**Table 5.** Nutritional composition of zeolite

Items	Zeolite
Clinoptilolite of sediment origin (%)	≥80
Clay minerals (%)	≤20
Particle size (mm)	0.01–0.2
Loss on drying (%)	≤6
SiO <sub>2</sub> (%)	62–73
Al <sub>2</sub> O <sub>3</sub> (%)	11–14
Si:Al ratio	4.8–5.4
CaO (%)	2–5.5
Na <sub>2</sub> O (%)	0.2–1.5
Fe <sub>2</sub> O <sub>3</sub> (%)	0.7–2.3
K <sub>2</sub> O (%)	2.2–3.4
MgO (%)	0.5–1.2
TiO <sub>2</sub> (%)	0.1–0.3

## RESULTS AND DISCUSSION

The average binding and buffering capacity and initial pH, are shown in Tables 6–9. Initial pH values ranged from 5.42 (lysine) to 6.75 (soybean extracted meal). Threonine had the highest buffering and binding capacity (602.60 meq/kg and 1519.49 meq/kg) and the lowest was maize (63.94 meq/kg and 232.97 meq/kg, respectively). The buffering capacity of the feed can influence the pH of the stomach and also the amount of HCl that is needed to acidify the stomach content (Mennah-Govela et al., 2019).

The ability of a feed item to withstand a change in pH value is known as acid binding capacity, and it can be used to determine the buffering capacity of feed ingredients. The values of buffering and binding capacity are comparable resp. there are slight differences stated by Lawlor et al. (2005). The milliequivalents (meq) of acid or base required to adjust the pH of the feed component to the pH end-titration are used to measure the acid binding capacity.

The pH in the stomach is more effectively neutralized by feed components with a high acid binding capacity than by feed components with a low acid binding capacity. The amount of crude protein and ash in the feed can affect its ability to bind acids (Lawlor et al., 2005).

In the feed mixture with the addition of zeolite, lower values of the initial pH and also lower values of the buffering activity were recorded. The addition of Zeolite to the feed mixture had a significant effect on the reduction of initial pH ( $P<0.001$ ), buffering ( $P<0.05$ ) and binding capacity ( $P<0.05$ ). There are more than 40 different types of zeolites that are naturally occurring silicate minerals, with clinoptilolite likely being the most prevalent. The acidity of the solution is typically increased by natural zeolites. (Inglezakis et al., 2003). The results showed that the buffering capacity of the analyzed feed mixtures is higher than the sum of the calculated buffering capacities of the individual feed components forming the feed mixtures. This means that the buffering capacities of the individual feed components do not have a cumulative effect.

**Table 6.** Acid-binding and buffering capacity of individual feed components

Items	SBM (n=6)	Corn (n=6)	Barley (n=6)	Wheat (n=6)	Lys (n=6)	Met (n=6)	Thr (n=6)
BC	271.08±5.01	63.94±2.67	92.37±14.3	72.25±5.28	311.51±4.37	431.31±14.33	602.6±43.36
ABC	1018.24±12.67	232.97±11.6	305.98±50.29	266.2±22.98	752.78±11.2	1230.7±45.96	1519.49±0.18
pH	6.75±0	6.64±0.01	6.28±0.04	6.67±0.02	5.42±0.03	5.85±0.05	5.53±0.18

Values are Means ± SD; BC – buffering capacity; ABC – acid binding capacity; SBM – soybean meal; Lys – Lysine, Met – Methionine; Thr – Threonine

**Table 7.** Acid-binding and buffering capacity of feed mixtures of control and experimental group

Items	Experimental (n=6)	Control (n=6)	P-value
BC (meq/kg)	192.08±9.67	201.64±2.9	*
ABC (meq/kg)	645.97±30.37	672.79±11.31	*
pH	6.32±0.01	6.38±0.01	***

Values are Means ± SD; BC – buffering capacity; ABC – acid binding capacity; \*P<0.05; \*\*\*P<0.001

**Table 8.** Acid-binding and buffering capacity of commercial mineral supplement and zeolite

Items	Commercial mineral supplement (n=6)	Zeolite (n=6)
BC (meq/kg)	4108.85±255.6	72.01±6.8
ABC (meq/kg)	11649.35±787.23	246.49±23.21
pH	5.82±0.13	6.43±0.05

Values are Means ± SD; BC – buffering capacity; ABC – acid binding capacity

**Table 9.** Buffering capacity (meq/g of feed) of feed mixture of control and experimental group

Items	BC experimental group	BC control group
Corn (%)	22.38 meq/350g	22.38 meq/350 g
Wheat (%)	13.01 meq/ 180 g	14.45 meq/200 g
Barley (%)	16.39 meq/ 177.4 g	16.39 meq/ 177.4g
Sybean meal (%)	65.06 meq/240 g	65.06 meq/24 g
Commercial mineral supplement (%)	6.05 meq/ 30 g	6.05 meq/ 30 g
Lysine (L-Lysine HCl) 78% (%)	0.31 meq/1 g	0.31 meq/1 g
Methionine (DL) (%)	0.22 meq/0.5 g	0.22 meq/0.5 g
Treonine (L) 98% (%)	0.66 meq/1.1 g	0.66 meq/1.1 g
Zeolite	1.44 meq/20 g	-
Calculated values of the BC	125.52 meq/1000 g	125.97 meq/1000 g
Analysed values of the BC	192.08 meq/1000 g	201.64 meq/1000 g

BC – buffering capacity

This fact was also confirmed by Moharrery (2007) in his study. A positive linear correlation was confirmed between the content of crude protein and the buffering capacity ( $P < 0.05$ ;  $r = 0.868$ ) and also between the ash and the buffering capacity ( $P < 0.01$ ;  $r = 0.9406$ ) (Figures 1 and 2).

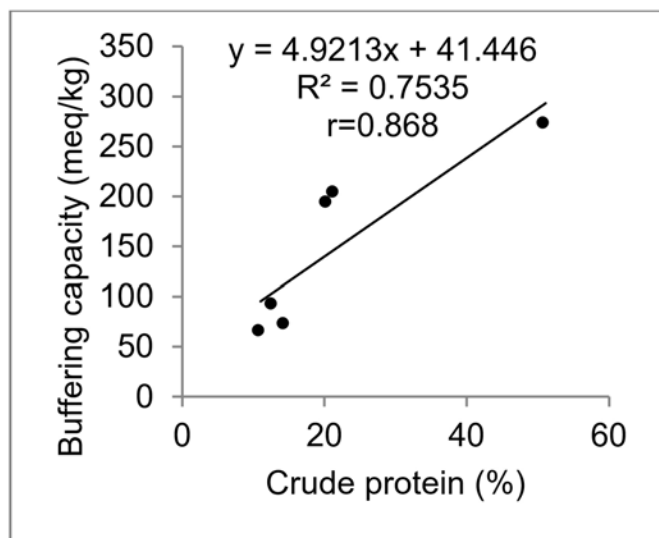


Figure 1. Relationship between buffering capacity and crude protein

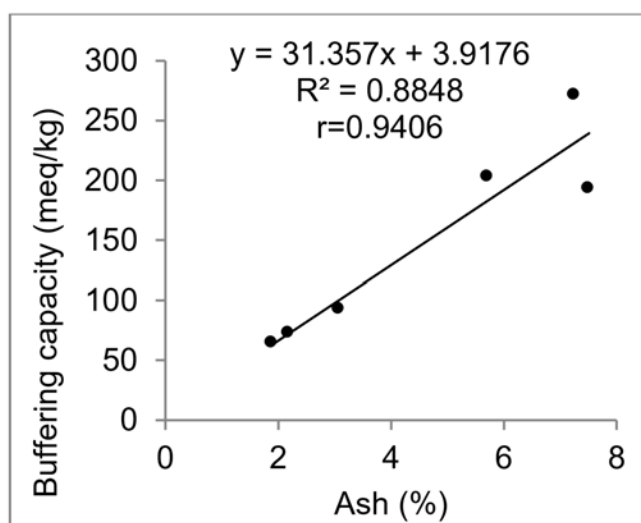


Figure 2. Relationship between buffering capacity and ash

The conclusions of Lawlor et al. (2005) and Huting et al. (2021) concur with these findings, according to which feed components with a higher content of nitrogenous and mineral substances (e.g. soybean meal, fish meal) have higher values of buffering capacity than,

for example, cereals. In addition to raising the likelihood of post-weaning diarrhea and perhaps affecting gastric barrier function, high stomach pH levels also increase the quantity of undigested protein that reaches the intestinal tract (Heo et al., 2013; Warneboldt et al., 2016; Huting et al., 2021). The benefits of adding acids to diets may come from maintaining a low gastric pH, which may improve protein digestion and decrease pathogen survival (Kim et al., 2005; Che et al., 2012; Huting et al., 2021). The ability to pick ingredients that are good for young piglets and to explain the buffering capacity of the entire diet can both be aided by knowledge of the buffering and binding capacities of each feed component in the diet.

## CONCLUSION

When monitoring the relationship between capacity and the number of nitrogenous substances and ash, a significantly increased buffering activity was recorded for feed components with a higher content of nitrogenous substances and ash than for other components with lower values of nitrogenous substances and ash. It was for those feed components that contained higher values of ash and crude protein that higher values of buffering and binding capacity were recorded. The feed mixture where the zeolite was added had a lower value of buffering activity than the feed mixture without the addition. The cumulative effect of the buffering capacities of the individual feed components forming the feed mixtures has not been demonstrated.

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