


INVESTIGATIONS ON PHYTATE DEGRADATION OF RAPESEED MEAL AND SOYBEAN MEAL IN RUMINANTS

YUNG-PING CHI

Investigations on phytate degradation of rapeseed meal and soybean meal in ruminants

Dissertation to obtain the doctoral degree of Agricultural Sciences

(Dr. sc. agr.)

Faculty of Agricultural Sciences

University of Hohenheim

Institute of Animal Science

submitted by

Yung-Ping Chi

from Pingtung City, Taiwan

2023

Die vorliegende Arbeit wurde am 26.01.2024 von der Fakultät Agrarwissenschaften der Universität Hohenheim als „Dissertation zur Erlangung des Grades eines Doktors der Agrarwissenschaften“ angenommen.

Tag der mündlichen Prüfung:	15.03.2024
Dekan:	Prof. Dr. Ralf Vögele
Leitung des Kolloquiums:	Prof. Dr. Jörn Bennewitz
Berichterstatter, 1. Prüfer:	Prof. Dr. Markus Rodehutscord
Berichterstatter, 2. Prüfer:	Prof. Dr. Fenja Klevenhusen
3. Prüfer:	Prof. Dr. Jana Seifert

This work was supported by the Hohenheim University Foundation

following a donation from alumni Dr Gerster

which is gratefully acknowledged.

TABLE OF CONTENTS

I LIST OF FIGURES

II LIST OF TABLES

III LIST OF ABBREVIATIONS

AA	Amino acid
CNCPS	Cornell Net Carbohydrate and Protein System
A	Non-protein nitrogen
B_1	Rapidly degradable protein fraction
B_2	Intermediate degradable protein fraction
B_3	Slowly degradable protein fraction
C	Unavailable protein fraction
CP	Crude protein
CPED	Rumen effective degradation of CP
DM	Dry matter
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
GfE	Gesellschaft für Ernährungsphysiologie
InsP	*Myo*-inositol phosphate
$InsP_1$	*Myo*-inositol monophosphate
$InsP_2$	*Myo*-inositol diphosphate
$InsP_3$	*Myo*-inositol triphosphate
$InsP_4$	*Myo*-inositol tetrakisphosphate
$InsP_5$	*Myo*-inositol pentakisphosphate
$InsP_6$	*Myo*-inositol hexakisphosphate
$InsP_6$-P	Phosphorus bound in $InsP_6$
$InsP_6$ED	Rumen effective degradation of $InsP_6$
$InsP_6ED_{2,5,6,8}$	Rumen effective degradation of $InsP_6$ at a rumen passage rate of 0.02, 0.05, 0.06, or 0.08 h^{-1}
$InsP_6$ED(mg)	$InsP_6$ED expressed as amount
$InsP_6$ED(%)	$InsP_6$ED expressed in percentage
Mg	Magnesium
MI	*Myo*-inositol
NRC	National Research Council
P	Phosphorus
Phytase	*Myo*-inositol 1,2,3,4,5,6 hexakisphosphate phosphohydrolases
PSV	Protein storage vacuole
r	Pearson correlation coefficient
R^2	Coefficients of determination
RUP	Rumen undegradable protein
RUSITEC	Rumen simulation technique
RSM	Rapeseed meal
SBM	Soybean meal
TiO_2	Titanium dioxide

1 GENERAL INTRODUCTION

Oilseeds contain not only valuable lipids which are important for human consumption but the processing by-products after oil extraction are also relevant protein sources for animal nutrition. These protein-rich plants and their by-products have reinforced increasing attention and interest since animal-based protein sources are restricted for livestock feeding due to safety concerns while the demand for livestock products such as meat and milk consumption increases rapidly in response to the population growth, particularly in the developing countries (FAO, 2021).

Over the decades, soybean meal (SBM) is the primary plant-based protein feed used for livestock to promote growth and production owing to its relatively balanced and available amino acid (AA) profile and its palatability. In 2019–2020, SBM used to feed livestock in the European Union (EU) reached 30 million tons according to EU-commission. Nevertheless, most of SBM used in the EU is imported from North- and South America despite a rapidly growing organic soy area in Central Europe, which not only had negative impact on the tropical biome due to the expanded soybean farming (Nepstad et al., 2014; Song et al., 2021) but also increases the carbon footprint during transportation (Escobar et al., 2020) and costs of animal production (Karlsson et al., 2021). As highlighted in the EU's Farm to Fork Strategy, a more sustainable and environmentally friendly agri-food systems should be established. This can be reached, for example, through providing animals with more locally grown and produced feed.

In Europe, rapeseed is the dominant oilseed crop and its production comprises almost 25% of the global production, with France as the leading producer, followed by Germany and Poland. According to EU Commission, around 13 million tons of rapeseed meal (RSM) were produced in the year 2022 and 76% thereof were used with rapeseed crop originated from the EU[1]. Rapeseed meal contains 30–40% of crude protein (CP) and the milk yield of lactating cows by supplying RSM was found to be comparable with SBM (Martineau et al., 2013). These conditions make RSM a

[1] EU Feed Protein Balance Sheet downloaded from: https://agriculture.ec.europa.eu/data-and-analysis/markets/overviews/balance-sheets-sector/oilseeds-and-protein-crops_en

promising alternative protein source to SBM in terms of meeting the growing demand for protein feed.

However, in addition to the high content of CP, RSM and SBM also contain high amount of phosphorus (P) in organic salt form, which is known as phytate. Although ruminants can efficiently utilise phytate-P, phytate may not be fully degraded and P not fully available to ruminants. Extending knowledge about effects of feeding diets containing different type of oilseed meals and their amounts on phytate degradation is necessary for a more accurate formulation of ruminant diets and an efficient supply of P in the future. The main objective of the present doctoral thesis was to investigate phytate degradation of RSM and SBM in ruminants, including their possible affecting factors.

2 LITERATURE REVIEW

Phosphorus is the second most abundant mineral which is involved in enzymatic functions, energy transfer, cell membrane structure and bone as well as soft tissue formation in the body. In ruminants, absorption of P occurs partially in the forestomach (Breves and Schröder, 1991) but mainly in the duodenum and jejunum of ruminants which is mediated by intestinal 1,25-$(OH)_2D_3$ receptor (Bruce et al., 1966; Pfeffer et al., 1970; Wilkens and Muscher-Banse, 2020) under modulation of dietary and endogenous P (Care, 1994). About 80% of absorbed P is deposited in the bone and teeth, and the remaining proportion is found in the body fluids and soft tissues. The homeostasis of P involves coordinated regulation of P absorption, excretion, and exchange of P between blood and bone or soft tissue (Wilkens and Muscher-Banse, 2020). Deficiency of P has been reported to reduce growth and bone stability of animals (Becker et al., 1933; Black et al., 1943; Shupe et al., 1988) and impair reproduction of lactating ruminants (Black et al., 1943).

In addition to the importance of P for animals, the symbiotic microorganisms in the rumen have a specific P requirement. Rumen microorganisms incorporate a remarkable amount of P as the component of nucleic acids and phospholipids, and require P for digestion of fiber (Durand and Komisarczuk, 1988) and synthesis of microbial protein (Zain et al., 2010). Therefore, insufficient P supply can also impair the growth and metabolism of rumen microorganisms, which consequently decreases the microbial fermentation and production of milk protein derived from microbial protein.

While extremely low dietary P causes deficiency symptoms and decreases production of animals, overfeeding of P does not further improve reproduction performance of animals (Lopez et al., 2004) but brings about environmental problem. Once the P requirement is met, the excess of P would be excreted primarily through faeces by ruminants, which has been found to linearly increase with higher intake of P (Wu et al., 2001, 2000). The excreted P from farmland accumulates in soil and water, endangering aquatic ecosystems as a consequence of eutrophication (Correll, 1998; Schindler et al., 2016).

To meet the maintenance and production requirement while minimise excess of P, Gesellschaft für Ernährungsphysiologie (GfE, 2001) and National Research Council (NRC, 2001) suggest a ration for high producing cows containing 0.35–0.40% of P on

a dry matter (DM) basis. The updating recommendation of GfE (2023) proposed that P in ration for lactating cows should be supplied under consideration of obligatory P losses, milk P content, and P deposition for maternal growth and in fetus. However, current dairy production systems tend to supply P in excess of these recommendations, partially resulted from additional supplementation of mineral P owing to the concern of marginal safety. With the knowledge about availability of P from plant feeds, it might be helpful for optimising the P utilisation and reducing the supplementation of mineral P.

In mature seeds, phytate, the salt form of *myo*-inositol 1,2,3,4,5,6 hexakis dihydrogen phosphate ($InsP_6$), comprises the main storage compound of P. The concentration of $InsP_6$ can vary with genotype of plants (Mebrahtu et al., 1997; Rodehutscord et al., 2016), growing conditions (Ishiguro et al., 2006), harvesting time (Mebrahtu et al., 1997; Santana et al., 2012), and processing procedures (Anderson and Wolf, 1995). Besides, different analytical assays can affect $InsP_6$ determined from feedstuffs (Marolt and Kolar, 2021; Phillippy et al., 2015). For common SBM, P from $InsP_6$ accounts for 30%–60% of total P (Eeckhout and de Paepe, 1994; Haese et al., 2017b; Ravindran et al., 1994; Tahir et al., 2012). For RSM, 35–75% of total P originates from $InsP_6$ (Eeckhout and de Paepe, 1994; Haese et al., 2017b, 2017a; Tahir et al., 2012; Viveros et al., 2000). Compared to $InsP_6$, concentrations of $InsP_5$ and other less phosphorylated *myo*-inositol phosphates (InsPs) in SBM and RSM are generally much lower (Haese et al., 2017b; Pontoppidan et al., 2007). Among the $InsP_5$ isomers, Ins(1,2,4,5,6)P_5 has been found to be the most abundant isomer in oilseed meals, followed by Ins(1,2,3,4,5)P_5 and then by Ins(1,2,3,4,6)P_5, while concentration of Ins(1,3,4,5,6)P_5 is very low even when detected (Haese et al., 2017b; Pontoppidan et al., 2007). $InsP_4$ is usually detected at very low concentration or not detectable at all (Haese et al., 2017b; Park et al., 2002; Pontoppidan et al., 2007), and $InsP_3$ are generally not detectable in oilseed meals (Haese et al., 2022, 2017b; Park et al., 2002). Nevertheless, concentrations of $InsP_{3–5}$ tend to increase while concentration of $InsP_6$ tends to decrease when the intensity of oilseed processing increases (Haese et al., 2022, 2017b; Konishi et al., 1999; Pontoppidan et al., 2007). As reported by Haese et al. (2022), $InsP_6$ decreased from 25 to 23 g/kg DM but $InsP_5$ and $InsP_4$ increased from 4.6 to 6.2 g/kg DM and from 0.35 to 0.95 g/kg DM, respectively, when heating time was increased from 48 to 93 min.

Phosphorus must be cleaved from the $InsP_6$ molecule to be absorbed by the intestinal epithelial cells and hence availability of $InsP_6$-P from feed depends on degradation of $InsP_6$ in the digestive tract prior to intestinal absorption (Bravo et al., 2003; Qian et al., 1997). The degradation of $InsP_6$ is accompanied by the formation of less phosphorylated InsPs and the completely proceeded cascade of P cleavage from $InsP_6$ terminates with the release of six phosphate groups and *myo*-inositol (MI), which can also be absorbed and utilised by animals (Huber, 2016). This stepwise dephosphorylation of $InsP_6$ is initialised by a type of phosphatase, specified as phytases (*myo*-inositol 1,2,3,4,5,6 hexakisphosphate phosphohydrolases) (Greiner and Konietzny, 2005). Phytases and other phosphatases have been found in plants, microorganisms and mucosa of gastrointestinal tract (Bitar and Reinhold, 1972; Eeckhout and de Paepe, 1994; Hu et al., 1996; Mitchell et al., 1997; Yanke et al., 1998). However, phytases produced by anaerobic rumen bacteria are regarded as the main contributor to $InsP_6$ degradation in ruminants (Lan et al., 2002b; Morse et al., 1992; Raun et al., 1956; Yanke et al., 1998). By screening 101 rumen bacteria cultures on $InsP_6$-containing plates, Yanke et al. (1998) determined phytase activity from bacterial strains of *Prevotella ruminicola*, *Megasphaera elsdenii*, *Selenomonas ruminantium*, *Mitsuokella multiacidus* and *Treponema* spp.. Lan et al. (2002b) further isolated the phytase-producing bacterial species *Mitsuokella jalaludinii* from the rumen and Nakashima et al. (2007) detected phytase sequences related to protein tyrosine phosphatases in *Selenomonas lacticifex*. Recent works isolating rumen bacteria with phytase activity also characterised the conditions for their growth and enzyme production or activity. For example, Lamid et al. (2018) found that rumen bacteria *Actinobacillus* sp., and *Bacillus vallimortis* have an optimal phytase activity between 45–50°C while *Bacillus pumilus* has an optimum between 40–45°C, with pH 4–5 as optimum for phytase production. Huang et al. (2011) cloned two most abundant cysteine phytase genes of goat rumen content and determined pH optima of these cysteine phytases similar to the typical rumen environment (pH 6.5 and 6.0). Moreover, Mootapally et al. (2016) identified the functional genes of phytase (RPHY1) from rumen *Prevotella* spp. and observed an optimal phytase activity at 55°C (pH 5) with a high stability at 5°C within the acidic pH range. The RPHY1 phytase activity was also found to be highly stimulated in the presence of EDTA but inhibited by metal ions. Combining the results from the aforementioned literature, it seems that characteristics

of phytase production by rumen bacteria vary between strains and the phytase activity can be induced or depressed by different factors.

Previously, ruminants were considered to be capable of utilising nearly all P bound in $InsP_6$ (Clark et al., 1986; Morse et al., 1992; Nelson et al., 1976). Based on faecal collection, Nelson et al. (1976) and Morse et al. (1992) found a total tract disappearance of $InsP_6$ greater than 99% in calves and lactating cows, respectively. The authors suggested that $InsP_6$ degradation is initiated by rumen microorganisms and proceeds to almost complete before digesta reaches the lower part of the digestive tract. Nevertheless, recent studies have shown a less complete $InsP_6$ disappearance determined from faeces, which was 69–98% in the study by Kincaid et al. (2005) and 85–94% in the study by Haese et al. (2014). Besides, ruminal $InsP_6$ disappearance varied considerably between and within studies. Haese et al. (2020) incubated different concentrate feeds in rumen fistulated cows and found the lowest rumen effective degradation of $InsP_6$ ($InsP_6$ED) for RSM (59%) but the highest for maize and faba beans (both 93%) at a rumen passage rate of 0.05 h^{-1}. Ray et al. (2013) fed lactating cows with different levels of dietary $InsP_6$ and observed 85–94% ruminal $InsP_6$ disappearance. In the *in situ* study by Haese et al. (2022), 26–52% of $InsP_6$ from RSM with different desolventising/toasting conditions was calculated to leave the rumen at a rumen passage rate of 0.05 h^{-1}. Different factors like concentrate type (Haese et al., 2020), $InsP_6$ amount in the diet (Ray et al., 2013), feed processing (Haese et al., 2022), and forage-to-concentrate ratio (Yanke et al., 1998) seem to influence the extent of ruminal $InsP_6$ degradation.

However, it is difficult to draw a general conclusion on the effects of different factors in ruminants due to the different conditions among studies. It is also not well known how ruminal $InsP_6$ degradation is affected by different factors and their combinations. Furthermore, studies regarding post-ruminal $InsP_6$ degradation are scarce. In case that ruminal $InsP_6$ degradation of dietary $InsP_6$ is not complete, post-ruminal $InsP_6$ degradation might be highly relevant. This doctoral thesis aimed to systematically study $InsP_6$ degradation of RSM and SBM in ruminants. Different study methods (*in vivo*, *in situ*, and *in vitro*) were combined to evaluate effects between RSM and SBM.

3 OVERVIEW OF INCLUDED EXPERIMENTS AND MANUSCRIPTS AND OBJECTIVES OF THE STUDIES

The studies in the present thesis were conducted with animals housed, fed and sampled according to German animal welfare regulations and the use was approved by the Regierungspräsidium (Stuttgart, Germany).

In Manuscript 1 (published in *Archives of Animal Nutrition*), RSM and SBM were incubated in the rumen of lactating Jersey cows for 2, 4, 6, 8, 16, 24, 48, and 72 h to obtain *in situ* data of $InsP_6ED$ for both oilseed meals at rumen passage rates of 0.02 ($InsP_6ED_2$) and 0.05 h^{-1} ($InsP_6ED_5$), and also to link these data with preliminary results from *in situ* studies conducted in cows. Secondly, a diet containing equal amount of RSM or SBM was fed to eight wethers for 8 weeks of adaptation. Titanium dioxide (TiO_2) was added at the end of the adaptation period to measure $InsP_6$ disappearance. Digesta from the reticulo-rumen (separated into 3 phases: large particulate matter, small particulate matter, and fluid phase), omasum, abomasum, jejunum, middle to posterior part of the colon, and rectum were collected and analysed for TiO_2, InsPs, and MI. The objective of Manuscript 1 was to investigate ruminal and post-ruminal $InsP_6$ degradation in wethers fed a diet containing RSM or SBM, and to compare the ruminal degradation with *in situ* results.

In Manuscript 2 (published in *Journal of Dairy Science*), 17 SBM variants from Europe, South and North America, and India were incubated with the same *in situ* procedure as carried out in Manuscript 1, and were tested for ruminal CP and AA degradation. Chemical protein fractionation of the 17 SBM samples was also conducted and was based on Cornell Net Carbohydrate and Protein System (CNCPS). Nine SBM samples with different rumen effective degradation of CP (CPED) were used to investigate the ruminal $InsP_6$ degradation. As $InsP_6$ in seeds is located in a protein-rich structure and $InsP_6$ degradation was reported to vary in a pattern similar to CP degradation for RSM in a recent study (Haese et al., 2022), Pearson correlation was performed to estimate the relationship between $InsP_6$ and CP degradation of SBM. The objective of Manuscript 2 was to determine the variation of *in situ* ruminal CP, AA, and $InsP_6$ degradation from commercial SBM, and to evaluate the possibility of predicting $InsP_6$

degradation from CP degradation and CNCPS protein fractions based on linear regression.

In the *in vitro* study (Titel: *in vitro* ruminal phytate degradation of rapeseed meal and soybean meal as affected by the provided amount of phytate), the same batches of RSM and SBM as used in Manuscript 1 were incubated with different amounts of $InsP_6$ for 3, 6, 12, 24, and 48 h in a modified rumen simulation technique (RUSITEC) system. The study was conducted because $InsP_6$ degradation from different feedstuffs has been evaluated mainly by including a certain amount of test feeds for incubation (Haese et al., 2020, 2017b; Morse et al., 1992) or for diet formulation (Manuscript 1) without considering different $InsP_6$ concentrations of the feedstuffs, and $InsP_6$ degradation is commonly expressed as relative value of $InsP_6$ in a feed such as percentage or g/kg of DM (Brask-Pedersen et al., 2011; Haese et al., 2020; Martín-Tereso et al., 2009; Park et al., 1999; Ray et al., 2013). Such approach may cause confounding with the amount of supplied $InsP_6$ and might not reflect the quantity of degraded $InsP_6$. In Manuscript 1, a higher ruminal $InsP_6$ disappearance was determined in wethers fed a diet containing SBM compared to those fed a diet containing the same amount of RSM, whereas a lower ruminally degraded amount of $InsP_6$ was observed upon feeding SBM. Thus, the objective of this study was to achieve a better understanding of how $InsP_6$ degradation of RSM and SBM is influenced by different amounts of $InsP_6$ in the feed and whether a limit of $InsP_6$ degradation exists.

4 INCLUDED MANUSCRIPTS AND STUDIES

4.1 MANUSCRIPT 1

Ruminal and post-ruminal phytate degradation of diets containing rapeseed meal or soybean meal

Yung-Ping Chi[1], Eva Haese[2], and Markus Rodehutscord[1]

[1]Institute of Animal Science, University of Hohenheim, Stuttgart, Germany

[2]Institute of Animal Science, University of Bonn, Bonn, Germany

Published in the Archives of Animal Nutrition (2022), 76: 233-247

The original publication is available at

https://doi.org/10.1080/1745039X.2022.2164158

DOI: 10.1080/1745039X.2022.2164158

ARCHIVES OF ANIMAL NUTRITION
2022, VOL. 76, NOS. 3–6, 233–247
https://doi.org/10.1080/1745039X.2022.2164158

Taylor & Francis
Taylor & Francis Group

Ruminal and post-ruminal phytate degradation of diets containing rapeseed meal or soybean meal

Yung-Ping Chi, Eva Haese* and Markus Rodehutscord

Institute of Animal Science, University of Hohenheim, Stuttgart, Germany

ABSTRACT

This study aimed to investigate ruminal and post-ruminal degradation of phytic acid ($InsP_6$) in diets containing either rapeseed meal (RSM) or soybean meal (SBM). In Experiment 1, the effective degradability of crude protein (CPED) and $InsP_6$ ($InsP_6ED$) was evaluated by incubating RSM and SBM *in situ* in three rumen-fistulated lactating Jersey cows for 2, 4, 6, 8, 16, 24, 48 and 72 h, and calculating effective degradability at rumen passage rates of 2% and 5%/h. In Experiment 2, eight wethers were assigned for 8 weeks to two dietary treatments (Diet RSM and Diet SBM) containing 150 g of either meal and 100 g of maize silage per feeding time and had free access to hay and water. Titanium dioxide (TiO_2) was added to the diets for the last 5 days of the study. The wethers were then stunned, exsanguinated and digesta from the reticulo-rumen, omasum, abomasum, jejunum, colon, and rectum were sampled. In Experiment 1, the $InsP_6ED$ of RSM ($InsP_6ED_2$: 83%; $InsP_6ED_5$: 64%) decreased almost identically to that of CPED with increasing passage rate ($CPED_2$: 78%; $CPED_5$: 63%) and was significantly lower than that of SBM ($InsP_6ED_2$: 93%; $InsP_6ED_5$: 85%). In Experiment 2, ruminal $InsP_6$ disappearance was significantly higher in wethers fed Diet SBM (89%) than in those fed Diet RSM (76%). Total post-ruminal $InsP_6$ degradation was 6% for Diet RSM and 4% for Diet SBM ($p = 0.186$). The total tract $InsP_6$ disappearance was higher in Diet SBM (93%) than in Diet RSM (82%). Considering higher $InsP_6$ contents in RSM, Diet RSM resulted in significantly higher amounts of ruminally (Diet RSM: 4.5 g/d; Diet SBM: 3.4 g/d) and total tract (Diet RSM: 4.9 g/d; Diet SBM: 3.5 g/d) degraded $InsP_6$. $InsP_5$ was quantified in most of the digesta samples after feeding Diet RSM but was not detectable in the majority of digesta samples for Diet SBM. Concentrations of *myo*-inositol (MI) tended to be higher ($p = 0.060$) in the blood plasma of wethers fed Diet RSM. The consistency between ruminal $InsP_6$ disappearance in wethers and *in situ* calculated $InsP_6ED_2$, along with the very low extent of post-ruminal $InsP_6$ degradation, suggests that at a low rumen passage rate, $InsP_6$-P from the feed becoming available to ruminants is almost entirely from $InsP_6$ degradation in the rumen.

ARTICLE HISTORY
Received 11 August 2022
Accepted 15 December 2022

KEYWORDS
$InsP_6$ degradation; rapeseed meal; soybean meal; inositol phosphates; *myo*-inositol; ruminants

CONTACT Markus Rodehutscord inst450@uni-hohenheim.de
*Present address: Institute of Animal Science, University of Bonn, Bonn, Germany

1. Introduction

Phytate [any salt of myo-inositol 1,2,3,4,5,6 hexakis (dihydrogen phosphate), $InsP_6$] represents the predominant phosphorus (P) source in plant seeds and their processing by-products. Phosphorus must be cleaved from $InsP_6$ before absorption. The cascade of P cleavage can proceed completely such that *myo*-inositol (MI), which can also be absorbed by animals, is released (Huber 2016). Such hydrolysis is catalysed by phytases and other phosphatases found in plants, microorganisms, and the mucosa of the intestine (Bitar and Reinhold 1972; Eeckhout and De Paepe 1994; Yanke et al. 1998).

Ruminants have been reported to be able to utilise almost all P bound to $InsP_6$ (Nelson et al. 1976; Morse et al. 1992) owing to the phytase activity of the rumen microbiota (Raun et al. 1956; Yanke et al. 1998). According to Nelson et al. (1976), degradation of $InsP_6$ is initiated in the rumen and proceeds to near completion before the digesta reaches the lower parts of the digestive tract. However, some recent studies have indicated that ruminal $InsP_6$ degradation is not complete, and the values vary between and within studies. In an *in situ* study by Haese et al. (2022), the ruminal escape of $InsP_6$ from rapeseed meal (RSM) was calculated to range from 26% to 52% at a rumen passage rate of 5%/h depending on the desolventising/toasting conditions that had been applied to the RSM. In a study by Park et al. (2002), more than 20% of dietary $InsP_6$ left the sheep rumen. With different levels of dietary $InsP_6$, Ray et al. (2013) observed 85–94% ruminal $InsP_6$ disappearance in first-lactation cows. Factors such as concentrate type (Haese et al. 2017a), feed processing (Haese et al. 2022), dietary $InsP_6$ concentration (Ray et al. 2013), and forage-to-concentrate ratio (Yanke et al. 1998) may affect the activity or accessibility of rumen microbial phytase and consequently the rate and extent of ruminal $InsP_6$ degradation.

Regarding the total digestive tract, faecal $InsP_6$ disappearance was reported to be 69–98% by Kincaid et al. (2005) and 85–94% by Haese et al. (2014). Nevertheless, *in vivo* studies describing ruminal and post-ruminal $InsP_6$ degradation, including the formation of lower inositol phosphate (InsP) isomers post-ruminally, are scarce. If $InsP_6$ from the feed cannot be completely degraded in the rumen, the relevance of post-ruminal $InsP_6$ degradation may be higher. The small and large intestines have been suggested to be relevant to post-ruminal $InsP_6$ degradation (Park et al. 2002; Ray et al. 2012, 2013; Jarrett et al. 2014).

The objective of this study was to investigate ruminal and post-ruminal $InsP_6$ degradation in wethers fed RSM- and soybean meal (SBM)-based diets, respectively. Previous *in situ* studies have shown a slower progression of $InsP_6$ disappearance in RSM compared to SBM (Haese et al. 2017a) and consequently a lower ruminal disappearance of $InsP_6$ for RSM than for SBM (Haese et al. 2020). Hence, we hypothesised lower ruminal and higher post-ruminal $InsP_6$ disappearance in wethers fed an RSM-containing diet than in those fed an SBM-containing diet. In addition, *in situ* ruminal $InsP_6$ disappearance was studied in rumen-fistulated cows in an attempt to link the data from the wether study with preliminary data from *in situ* studies conducted in cows.

2. Materials and methods

2.1. Experiment 1

An *in situ* study approved by the Animal Welfare Authority (Regierungspräsidium Stuttgart, Germany, approval code: V352/18 TE) was conducted according to German

animal welfare regulations. Rapeseed meal and SBM were incubated in three rumen-fistulated lactating Jersey cows. The cows were fed a total mixed ration (TMR) consisting of 35% concentrate mixture (16% winter barley, 21% maize, 27% faba beans, 16% peas, and 20% rapeseed cake), 2% RSM, 23% maize silage, 14% grass silage, 17% hay, and 2.5% straw on a dry matter (DM) basis. The content of P, net energy for lactation, and crude protein (CP) in the TMR was 4.43 g/kg DM, 6.7 MJ, and 149 g/kg DM, respectively. The average daily DM intake and milk yield of cows were 20 kg and 23 kg, respectively. Feed and water were provided for *ad libitum* consumption.

Rapeseed meal and SBM containing 21.6 g/kg DM and 14.2 g/kg DM $InsP_6$, respectively (Table 1), were incubated based on the recommended protocol of the GfE [Gesellschaft für Ernährungsphysiologie] (2022) and all details as described by Seifried et al. (2017). Briefly, SBM and RSM were ground to pass through a 2-mm sieve. An amount of 8 g (± 0.015 g) was weighed and placed into polyester bags (10 × 20 cm, pore size of 50 μm, ANKOM Technology, USA) and subsequently incubated in the rumen for 2, 4, 6, 8, 16, 24, 48, and 72 h. To acquire a sufficient amount of incubation residue, three replications for 2, 4, 6 and 8 h, four replications for 16 h, and six replications for 24, 48, and 72 h were incubated. The bags were immersed in warm water (approximately 39°C) for 10 min before placement in the rumen. Three bags of each meal were washed in a washing machine (Miele & Cie. KG, Gütersloh, Germany) without ruminal incubation to obtain the value at 0 h.

Following withdrawal from the rumen, bags were immediately soaked in ice-cold water to suppress microbial fermentation. The bags were then rinsed with cold tap water to remove adherent particles and subsequently washed in a washing machine (Miele & Cie. KG, Gütersloh, Germany) for 20 min without detergent and spinning. All the bags were then dried for 24 h at 60°C. The residues from the dried bags were weighed and pooled per cow and incubation time. Pooled samples were pulverised using a mixer mill (Type MM400, Retsch GmbH, Haan, Germany) and stored at 4°C until further processing.

Table 1. Analysed composition of single feeds and diets containing rapeseed meal (Diet RSM) or soybean meal (Diet SBM) [g/kg DM[#]].

	Rapeseed meal	Soybean meal	Maize silage	Hay
Crude protein	371	526	76	54
Crude ash	88	84	30	59
NDFom[†]	325	176	444	696
ADFom[§]	264	73	246	462
P	11.6	7.1	2.0	2.0
$InsP_6$	21.6	14.2	n.d.[◊]	n.d.
$InsP_5$[¶]	3.9	2.6	n.d.	n.d.
$InsP_4$[‡]	0.10	n.d.	n.d.	n.d.
Myo-inositol	0.30	0.65	1.88	0.44
	Diet RSM		Diet SBM	
$InsP_6$	17.7		11.6	
$InsP_5$	3.2		2.1	
Myo-inositol	0.57		0.86	
P	3.3		2.0	

Notes: [#]DM, dry matter; [†]NDFom, neutral detergent fibre expressed exclusive of residual ash; [§]ADFom, acid detergent fibre expressed exclusive of residual ash; [¶]sum of $InsP_5$ isomers; [‡]sum of $InsP_4$ isomers; [◊]n.d., not detectable.

2.2. Experiment 2

2.2.1. Animals and diets

The experiment was approved by the Animal Welfare Officer of the University of Hohenheim (approval code: T/192/19 TE) and was conducted in accordance with the German Animal Welfare Legislation. Eight 10-year-old Merino wethers (body weight: 110 ± 8 kg) were randomly assigned to two dietary treatments and kept in a pen with straw bedding.

Two experimental diets using RSM and SBM from the same batch as in Experiment 1 were freshly prepared (Diet RSM: 100 g maize silage + 150 g RSM; Diet SBM: 100 g maize silage + 150 g SBM) and fed twice daily at 7:30 and 16:00 h right after mixing. The concentration of $InsP_6$ was calculated to be 17.7 g/kg DM in Diet RSM and 11.6 g/kg DM in Diet SBM based on the concentrations in the single feeds except for hay (Table 1). To reduce selection and ensure complete feed intake, 30 ml water was added during mixing. The wethers had free access to hay and water. The diets were provided for 8 weeks to ensure sufficient adaptation time. In the first 6 weeks of adaptation, the wethers were kept in two separate groups according to their treatment and fed on a group basis. From week 7 onwards, the wethers were individually fed their respective diets. At the end of the adaptation period, titanium dioxide (TiO_2) was added to the diets (1.3 g per feeding time per animal).

Rapeseed meal, SBM, and hay were collected, stored at 4°C. Maize silage was frozen and lyophilised. Maize silage and hay samples were pooled over time to obtain one sample for each feed. The hay sample was first ground through a 2-mm sieve to obtain smaller feed particles (Type SM1, Retsch GmbH, Haan, Germany). Subsequently, all feed samples were ground to pass through a 0.5-mm sieve (Ultra Centrifugal Mill ZM 200, Retsch GmbH, Haan, Germany), and a portion of each was further pulverised using a mixer mill (Type MM400, Retsch GmbH, Haan, Germany).

2.2.2. Sampling and sample preparation

Samples were obtained on four separate days. On each sampling day, two wethers (one from each treatment group) were fed their respective diet 2 h before they were sacrificed and had no access to hay in that time period to achieve a similar rumen fill. The animals were stunned with a captive bolt gun and exsanguinated by cutting the jugular vein.

Blood from the *vena jugularis* was collected immediately after exsanguination into Sarstedt® collectors [Glucose FH; Sarstedt AG & Co., Nümbrecht, Germany] containing sodium fluoride and centrifuged at 2,000 × *g* for 10 min to separate the plasma. The left flank and abdominal wall musculature of the wethers were incised using a commercial blade. Digestive tract sections were separated and clamped using forceps. The reticulo-rumen contents were collected as rumen pools and pressed through a coarse Hessian bag. Particulate matter retained in the bag was weighed and defined as large particulate matter (LPM). Five percent of the total weight of the LPM was collected for analysis. The filtrate was collected in a bucket and immediately homogenised using a magnetic stirrer. Approximately 800 g of the filtrate was transferred to centrifuge cups and centrifuged at 10,000 × *g* at 4°C for 30 min. The supernatant was collected and defined as the fluid phase (FP), whereas the remaining pellet was resuspended in double-distilled water and centrifuged again. The supernatant from the second centrifugation step was discarded and the residue was harvested as small particulate matter (SPM). Digesta from the

omasum, abomasum, colon (from half of the spiral colon to 2 m before the entrance of the rectum), and rectum were quantitatively collected. Contents from 6 m posterior to the transition of the small intestine and 2 m prior to the transition of the large intestine were collected as jejunum samples, which were then rinsed with cold double-distilled water. Digesta samples were immediately frozen at −20°C after sampling or centrifugation. The frozen digesta samples were then lyophilised. Eight to 14 g of each dried digesta sample were pulverised using a mixer mill (Type MM400; Retsch GmbH, Haan, Germany) prior to chemical analysis.

2.3. Chemical analyses

Analysis of crude nutrients followed the official methods in Germany (Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten (VDLUFA) 2012). The feed samples were analysed for DM (method 3.1), crude ash (method 8.1), CP (method 4.1.1), crude fibre (method 6.1.1), neutral detergent fibre without residual ash (NDFom; method 6.5.1), and acid detergent fibre without residual ash (ADFom; method 6.5.2).

In the bag residues from Experiment 1, DM and CP were determined as described above. To determine the concentrations of $InsP_6$ in the concentrates and bag residues, samples were extracted using the method of Zeller et al. (2015) with modifications described by Sommerfeld et al. (2018). In brief, samples were extracted with a solution of 0.2 M EDTA and 0.1 M sodium fluoride at 4°C (pH = 8) for 30 min. After centrifugation following two extractions at 12,000 × *g* for 15 min, 1 ml sample was collected and centrifuged at 14,000 × *g* for 15 min and filtered prior to measurement by high-performance ion chromatography (ICS-3000 system, Dionex, Idstein, Germany).

In Experiment 2, concentrations of TiO_2 were determined in the pulverised digesta samples using a modified sulphuric and nitric acid wet digestion method of Boguhn et al. (2009), followed by measurement on an inductively coupled plasma optical emission spectrometer as described in detail by Zeller et al. (2015). To determine the concentrations of $InsP_{3\text{-}6}$ in the feed and digesta, samples were extracted using the method described in Experiment 1. To determine $InsP_{1\text{-}2}$ in the feed and digesta samples, a buffer solution containing 50 mM Tris, 50 mM glycine, and 0.2 M sodium fluoride at pH 9 was used for the extraction; otherwise, the same procedure for determining $InsP_{3\text{-}6}$ was performed. The concentrations of MI in the feed, blood plasma, and digesta samples were measured based on the method described by Sommerfeld et al. (2018) using gas chromatography/mass spectrometry after derivatisation of the samples. The analysed values of the omasum and abomasum were combined as omasum + abomasum based on their DM fractions for further calculations, as the complete separation of the omasal and abomasal digesta during sampling was not possible due to the exchange of fluids between these two sections.

2.4. Calculations and statistics

In Experiment 1, the equations suggested by Ørskov and McDonald (1979) [Equation (1)] and McDonald (1981) [Equation (2)] were used to describe ruminal degradation kinetics, and calculations were performed using GraphPad Prism software (version 5.0, GraphPad Software Inc., CA, USA):

$$Degradation\,[\%] = a + b \cdot (1 - e^{-ct}) \tag{1}$$

$$Degradation[\%] = a + b \cdot \left(1 - e^{-c(t-L)}\right) for\ t > L \tag{2}$$

where a [%] is the rapidly degradable fraction acquired from the 0 h incubation time, b [%] is the potentially degradable fraction, c [%/h] is the degradation rate, and L [h] represents the lag time. The best-fitting model for each feed was selected using the Akaike Information Criterion (AIC). The effective degradability (ED) of CP and $InsP_6$ was calculated using either the equation of McDonald (1981) [Equation (3)] or the modified equation of Wulf and Südekum (2005) [Equation (4)], assuming rumen passage rate (k) = 0.02 and 0.05 per h.

$$ED\,[\%] = a + [(b \cdot c)/(c + k)] \tag{3}$$

$$ED\,[\%] = a + [(b \cdot c)/(c + k)] \cdot e^{-kL} \tag{4}$$

In Experiment 2, $InsP_6$ disappearance was calculated based on the concentrations of $InsP_6$ and TiO_2 in the diet and digesta, using the following equation:

$$InsP_6 disappearance[\%] = 100 - 100 \cdot \left(\frac{TiO_2 indiet\left[\frac{g}{kg}DM\right]}{TiO_2 indigesta\left[\frac{g}{kg}DM\right]}\right) \cdot \left(\frac{InsP_6 indigesta\left[\frac{g}{kg}DM\right]}{InsP_6 indiet\left[\frac{g}{kg}DM\right]}\right)$$

where the TiO_2 concentration in the diet was calculated as the amount of TiO_2 provided [g/d] divided by the DM intake of the diet [kg/d].

Statistical analysis of the data was performed using the software program SAS (version 9.4, SAS Institute Inc., Cary, USA) using the SAS statement PROC MIXED with the following model:

$$y_i = \mu + \alpha_i + e_i$$

where y_i is the target trait, α_i is the fixed treatment effect, μ is the overall mean, and e_i is the residual error of y_i. Statistical significance was set at $p \leq 0.05$. Data are presented as the mean and standard error of the mean.

3. Results

3.1. Experiment 1

Fraction *a* of $InsP_6$ averaged 1.9% for RSM and 32% for SBM. Fraction *b* was 98% and 68% for RSM and SBM, respectively (Table 2). A lag time for $InsP_6$ degradation was observed at 3.6 h for RSM and 1.0 h for SBM. The degradation rate *c* was lower for RSM (16%/h) than for SBM (23%/h). Fractions *a* and *b* of CP were 13% and 82% for RSM and 10% and 90% for SBM, respectively. No lag phase in CP degradation was observed for either meal. The degradation rate *c* of CP was 7.9%/h for RSM and 8.4%/h for SBM, with

no significant differences. CPED was higher for SBM than for RSM ($CPED_2$: 83 vs. 78%; $CPED_5$: 66 vs. 63%). The RSM values of $InsP_6ED$ resembled those of CPED ($InsP_6ED_2$: 83%; $InsP_6ED_5$: 64%) and were significantly lower than those of SBM ($InsP_6ED_2$: 93%; $InsP_6ED_5$: 85%).

3.2. Experiment 2

3.2.1. Concentrations of inositol phosphate isomers and myo-inositol

In the FP of the rumen, $InsP_6$ concentration was lower than the limit of quantification (<0.13 g/kg DM) and $InsP_5$ was not detectable (Table 3). Feeding RSM resulted in significantly higher $InsP_6$ concentrations in the SPM (1.53 vs. 0.59 g/kg DM) and LPM (2.21 vs. 0.83 g/kg DM) of the rumen pool, omasum + abomasum (1.52 vs. 0.47 g/kg DM), colon (1.54 vs. 0.43 g/kg DM), and rectum (1.57 vs. 0.43 g/kg DM). $InsP_5$ was quantified in the SPM and LPM of the rumen pool, omasum + abomasum, jejunum, colon, and rectum of wethers fed Diet RSM but was not detectable in the majority of digesta samples from those fed Diet SBM. $InsP_{1\text{-}4}$ were not detected in any digesta samples.

Myo-inositol was not quantifiable (≤0.05 g/kg DM) in the majority of the samples from the rumen pool, colon, and rectum. Only traces of MI were determined, without significant differences in the omasum + abomasum (Table 4). The MI concentration in the jejunal content was not significantly different but higher for Diet RSM than Diet SBM by trend in the blood plasma (4.6 vs. 3.8 μg/ml).

3.2.2. $InsP_6$ disappearance and degraded amount of $InsP_6$

Ruminal $InsP_6$ disappearance measured at the omasum + abomasum was 89% for Diet SBM, which was significantly higher than that in Diet RSM (76%; Table 5). Up to the jejunum, 88% and 94% of ingested $InsP_6$ disappeared in wethers fed Diet RSM and Diet SBM, respectively. $InsP_6$ disappearance up to the colon and rectum was higher in the Diet SBM group than in the Diet RSM group ($p = 0.046$ and 0.057, respectively). Total post-

Table 2. Estimated ruminal degradation parameters and effective degradability of phytate ($InsP_6$) and crude protein (CP) from *in situ* incubation of rapeseed meal (RSM) and soybean meal (SBM).

	RSM	SBM	SEM[#]	*p*-Value
CP degradation				
$a^{\dagger}$ [%]	13	10	-	-
$b^{\S}$ [%]	82	90	-	-
$c^{\P}$ [%/h]	7.9	8.4	0.40	0.349
$CPED_2^{\ddagger}$ [%]	78	83	0.8	0.006
$CPED_5$ [%]	63	66	0.9	0.038
$InsP_6$ degradation				
$Lag^{\Diamond}$ [h]	3.6	1.0	0.30	0.005
a [%]	1.9	32	-	-
b [%]	98	68	-	-
c [%/h]	16	23	1.4	0.007
$InsP_6ED_2^{\blacklozenge}$ [%]	83	93	0.5	0.003
$InsP_6ED_5$ [%]	64	85	1.1	0.005

Notes: Data are presented as treatment means; $n = 3$ animals; [#]SEM, standard error of the mean; [†]*a*, rapidly degradable fraction; [§]*b*, potentially degradable fraction; [¶]*c*, degradation rate; [‡]CPED, effective degradability of CP at rumen passage rates of 2 ($CPED_2$) and 5 ($CPED_5$) %/h; [◊]Lag, lag time; [◆]$InsP_6ED$, effective degradability of $InsP_6$ at rumen passage rates of 2 ($InsP_6ED_2$) and 5 ($InsP_6ED_5$) %/h.

Table 3. Concentrations of inositol phosphate (InsP) isomers in digestive tract contents of wethers fed diets containing rapeseed meal (Diet RSM) or soybean meal (Diet SBM) [g/kg DM[#]].

	Diet RSM	Diet SBM	SEM[†]	*p*-Value
$InsP_6$				
Rumen pool				
Fluid phase	<LOQ[‡]	<LOQ	-	-
Small particulate phase	1.53	0.59	0.226	0.026
Large particulate phase	2.21	0.83	0.292	0.015
Omasum + abomasum[§]	1.52	0.47	0.206	0.011
Jejunum	0.67	0.23	0.177	0.131
Colon	1.54	0.43	0.236	0.016
Rectum	1.57	0.43	0.256	0.020
$InsP_5$[¶]				
Rumen pool				
Fluid phase	n.d.[◊]	n.d.	-	-
Small particulate phase	0.30	n.d.	-	-
Large particulate phase	0.36	n.d.	-	-
Omasum + abomasum	0.22	n.d.	-	-
Jejunum	0.05	n.d.	-	-
Colon	0.13	n.d.	-	-
Rectum	0.07	n.d.	-	-

Notes: Data are presented as treatment means, $n = 4$ animals; [#]DM, dry matter; [†]SEM, standard error of the mean; [§]Calculated with respective fractions [%] of omasal and abomasal digesta on a dry matter basis; [¶]The sum of $InsP_5$ isomers; [‡]<LOQ, not quantifiable; [◊]n.d., not detectable.

Table 4. Concentrations of *myo*-inositol in digestive tract contents [g/kg DM[#]] and blood plasma [μg/ml] of wethers fed diets containing rapeseed meal (Diet RSM) or soybean meal (Diet SBM).

	Diet RSM	Diet SBM	SEM[†]	*p*-Value
Omasum + abomasum[§]	0.05	0.02	0.018	0.386
Jejunum	0.99	0.72	0.120	0.162
Blood plasma	4.6	3.8	0.24	0.060

Notes: Data are presented as treatment means, $n = 4$ animals; [#]DM, dry matter; [†]SEM, standard error of the mean; [§]Calculated with respective fractions [%] of omasal and abomasal digesta on a dry matter basis.

ruminal $InsP_6$ disappearance, calculated as the difference between ruminal and total tract $InsP_6$ disappearance, did not differ between Diet RSM and Diet SBM (6 vs. 4%). A significantly higher amount of $InsP_6$ was degraded ruminally and in total tract for Diet RSM (ruminal: 4.5 g/d; total tract: 4.9 g/d) in comparison with Diet SBM (ruminal: 3.4 g/d; total tract: 3.5 g/d; Figure 1).

4. Discussion

4.1. Degradation characteristics of experimental feed determined in situ

The significantly lower $InsP_6$ED for RSM compared to SBM is consistent with the results from previous *in situ* studies (Park et al. 1999; Konishi et al. 1999; Haese et al. 2020). As suggested by the previous authors, this observation may be attributed to the differences in the location and binding form of $InsP_6$ in rapeseed and soybean, as these can influence the solubility of $InsP_6$ as well as the accessibility of microbial phytase to its substrate. Thus, the aggregation of $InsP_6$ with proteins inside protein storage vacuoles (Gillespie

Table 5. Ruminal and post-ruminal $InsP_6$ disappearance in wethers fed diets containing rapeseed meal (Diet RSM) or soybean meal (Diet SBM) [%].

	Diet RSM	Diet SBM	SEM[#]	*p*-Value
Ruminal[†]	76	89	3.2	0.033
Post-ruminal				
Jejunum	88	94	2.9	0.297
Colon	83	92	2.8	0.046
Rectum	82	93	3.0	0.057
Total post-ruminal[§]	6	4	1.2	0.186

Notes: Data are presented as treatment means, $n = 4$ animals; [#]SEM, standard error of the mean; [†]Calculated with respective $InsP_6$ disappearance in fractions of omasum and abomasum on a dry matter basis; [§]Calculated as the difference between total tract (rectum) and ruminal disappearance.

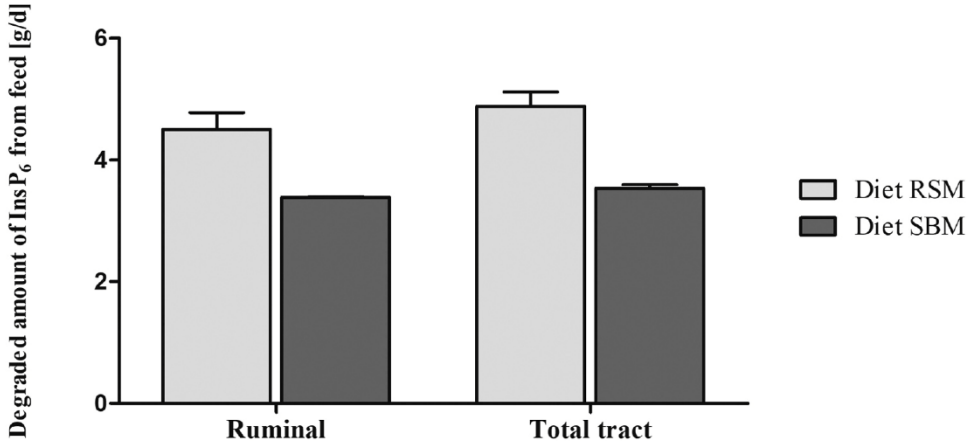

Figure 1. Amount of ruminal ($p = 0.007$) and total tract ($p = 0.002$) degraded $InsP_6$ from feed in wethers fed diets containing rapeseed meal (Diet RSM) or soybean meal (Diet SBM) [g/d]; data are presented as treatment means ($n = 4$ animals) with error bars (standard error of the mean) and calculated with differences between intake and omasum + abomasum (ruminal), and intake and rectum (total tract), respectively.

et al. 2005) and a higher amount of less soluble Mg- and Ca-phytate (Gillberg and Törnell 1976) compared to K-phytate may slow the degradation process in RSM, resulting in a greater rumen outflow of $InsP_6$ in comparison with SBM. An almost identical $InsP_6ED$ and CPED for RSM against a difference of approximately 20% points between $InsP_6ED_5$ and $CPED_5$ for SBM in the current study also confirms the close relationship between $InsP_6$ and CP degradation in RSM, as previously reported (Konishi et al. 1999; Haese et al. 2017a, 2022).

4.2. Ruminal $InsP_6$ disappearance

Voluntary hay intake of wethers may have been different and straw intake due to housing on straw bedding might have occurred. However, the hay did not contain $InsP_6$ (Table 1) and a previous study of our department found an $InsP_6$ concentration below 0.1 g/kg DM in straw (unpublished data). Assuming that the straw used in the present study contained $InsP_6$ at a similarly low level as in the previous study, the calculations of $InsP_6$

disappearance can be regarded as not affected by variable straw and hay intake of the wethers. Consistent with the relatively high $InsP_6$ content of Diet RSM compared to Diet SBM, $InsP_6$ concentrations determined in the particulate matter of the rumen pool and omasum + abomasum were also higher. However, the amount of ruminally degraded $InsP_6$ was greater for Diet RSM (4.5 g/d) than for Diet SBM (3.4 g/d) despite the lower ruminal disappearance for Diet RSM (76%) compared to Diet SBM (89%). This is consistent with the study by Ray et al. (2013), where a high content of $InsP_6$ in the feed stimulated microbial phytase activity in the rumen, leading to increased ruminal $InsP_6$ degradation. In the present study, the wethers were assumed to have a low rumen passage rate, as they were fed to meet maintenance energy requirements, and the measured ruminal $InsP_6$ disappearance was almost identical to the *in situ* calculated $InsP_6ED_2$ in Experiment 1. The extent of ruminal $InsP_6$ disappearance seems to be very high if the retention time of the digesta in the rumen is long. Accordingly, the relatively low ruminal $InsP_6$ disappearance (55–66%) reported by Kebreab et al. (2005) may be ascribed to a high passage rate in lactating cows fed high levels of whole crop wheat, as insufficient time of digesta retention in the rumen renders microbial hydrolysis of $InsP_6$ less complete. However, in the present study, despite a long ruminal retention time, $InsP_6$ disappearance was not complete, and a greater portion of $InsP_6$ from RSM than SBM remained undegraded by rumen microorganisms, as also characterised by Experiment 1. This is inconsistent with the finding that nearly 100% of $InsP_6$ is ruminally degradable (Nelson et al. 1976). In that study, the calves were fed a diet based on corn and SBM. A much faster disappearance (Haese et al. 2017a) and a higher $InsP_6ED$ for corn than for SBM have been reported (Haese et al. 2020), which may explain the differences between the current study and Nelson et al. (1976).

Notably, no $InsP_6$ was detected in the FP of the rumen pool in either treatment. In an *in vitro* study, the $InsP_6$ concentration in the fermenter fluid markedly decreased between 0 and 3 h of incubation (Haese et al. 2017b), indicating that the fraction of $InsP_6$ dissolved in the rumen fluid was rapidly degraded. This supports the assumption made in the *in situ* study that the soluble fraction *a* of $InsP_6$ determined by the washing procedure, is rapidly degraded. The fact that only traces of $InsP_5$ and no less phosphorylated InsP isomers were detected is consistent with the assertions of *in situ* (Haese et al. 2020) and *in vitro* studies (Brask-Pedersen et al. 2011) that the initial degradation step of $InsP_6$ determines the extent of degradation. Once the first phosphate group is cleaved from the molecule, degradation proceeds quickly and completely in ruminants, which deviates from non-ruminants, where the addition of exogenous phytase to the feed increases the concentrations of $InsP_4$ and less phosphorylated InsP isomers (Rodehutscord et al. 2022).

4.3. Post-ruminal $InsP_6$ disappearance

Total post-ruminal $InsP_6$ disappearance was 6% (Diet RSM) and 4% (Diet SBM) when expressed as a percentage of feed $InsP_6$ (equivalent to 25% for Diet RSM and 35% for Diet SBM as percentage of ruminal $InsP_6$ outflow). These values are relatively low compared to the values reported by Park et al. (2002) (17%) and Kebreab et al. (2005) (11–27%). If $InsP_6$ is not degraded, even after a long retention time of digesta in the rumen, it may not undergo substantial post-ruminal degradation. The greater extent of post-ruminal $InsP_6$ disappearance observed in the aforementioned studies may be a result of the degradation

of potential rumen degradable $InsP_6$ which was not entirely degraded before leaving the rumen due to an insufficient retention time of digesta. Ruminally undegraded $InsP_6$ was presumably mainly degraded in the upper part of the small intestine in the current study, as $InsP_6$ disappearance and increased MI concentrations were observed in the jejunum compared to the omasum + abomasum in both treatments. This is similar to the result of the study by Ray et al. (2013), where approximately 78% of post-ruminal $InsP_6$ degradation occurred between the omasum and ileum. In contrast, Park et al. (2002) reported that almost no $InsP_6$ was degraded between the abomasum and the jejunum. In that study, 88% of the post-ruminal $InsP_6$ degradation occurred posterior to the jejunum. Although intestinal CP degradation was not measured, it is highly possible that some of the ruminally undegraded $InsP_6$ was released from protein-phytate complexes through the enzymatic digestion of rumen undegradable protein (RUP) in the abomasum and upper part of the small intestine, which was subsequently subjected to degradation by phosphatases and phytases of microorganisms and the intestinal mucosa (Bitar and Reinhold 1972). A relatively higher extent of $InsP_6$ disappearance (0.7 vs. 0.2 g/d) observed between the omasum + abomasum and jejunum upon feeding Diet RSM may be attributed to a higher amount of $InsP_6$ entering from the rumen (1.4 vs. 0.4 g/d) or that released from RUP degradation through the intense association between $InsP_6$ and CP.

In total, 18% and 7% of dietary $InsP_6$ reached the rectum of wethers fed Diet RSM and Diet SBM, respectively. Apparently, rumen undegradable $InsP_6$ from the used meals is also resistant to microbial degradation in the large intestine. Similarly, Brask-Pedersen et al. (2013) found no further reduction of $InsP_6$-P posterior to the ileum (2.6 g/d in the ileum and 2.7 g/d in the faeces) in lactating cows fed TMR comprising 20% DM of rapeseed cake. Although the large intestine is the second most common site of microbial fermentation in ruminants, and certain phytase-producing microbes in the large intestine, similar to those in the rumen, are considered to contribute to large intestinal $InsP_6$ degradation (Ray et al. 2012, 2013; Jarrett et al. 2014), the large intestine is characterised by a lower fill capacity, a much shorter retention time, and a more homogenous digesta composition compared to the rumen (Mambrini and Peyraud 1997). A less efficient microbial phytase has been reported in the large intestine than in the rumen (Ray et al. 2012). In that study, less than 20% of the $InsP_6$ infused into the ileum was degraded in the large intestine, and this did not differ between the infusion rates of $InsP_6$. Wang et al. (2020) found that the number of bacterial genera significantly correlated with P digestibility was lower in the jejunum, followed by the colon, than in the forestomach of goats. This is reinforced by the results of Park et al. (2002), in which 76% of $InsP_6$ disappeared in the rumen, followed by 13% $InsP_6$ disappearance between the jejunum and colon. Combined with the results from this study and other studies, it can be concluded that a certain portion of potentially rumen degradable $InsP_6$ is degraded in the large intestine, whereas rumen undegradable $InsP_6$ remains mostly undegraded until it is excreted. Despite the great capability of ruminants to digest high amounts of $InsP_6$ from feed, as the total tract degraded amount of $InsP_6$ was 4.9 g/d for Diet RSM and 3.5 g/d for Diet SBM, processing of oilseeds leads to the formation of RUP, which may render $InsP_6$-P unavailable for ruminants.

4.4. Myo-inositol

Trace amounts of MI were detected in the omasum + abomasum, and no MI was detected in the rumen pool, colon, or rectum in either treatment group. In pigs, the digesta MI concentration was markedly reduced in the hindgut when they were fed corn-SBM- and corn-SBM-rapeseed cake-based diets with or without phytase supplementation (Rosenfelder-Kuon et al. 2020). The authors suggested the possibility of absorption by the large intestine or microbial degradation. It is not known whether MI can also be absorbed by the epithelial cells of the forestomach. However, it is very likely that the microbiota in the rumen metabolises the MI released from $InsP_6$. For archaea, bacteria, and eukaryotes, such as protozoa and fungi which are present in the forestomach (Castillo-González et al. 2014; Liu et al. 2021), MI serves as an essential component of the membrane and as a precursor for other molecules (Michell 2008; Reynolds 2009). In the current study, MI was quantified in the jejunum, where fewer microbes are active (Mao et al. 2015). In wethers fed Diet RSM, there was a higher proportion of ruminally undegraded InsP isomers, which provided more substrate for phosphatases and phytases in the upper small intestine, and thus might have led to the numerically higher MI concentration compared to Diet SBM. This is supported by the fact that the lowest $InsP_6$ concentration was found in the digesta of the jejunum.

The MI concentration in the blood plasma tended to be higher in wethers fed Diet RSM than in those fed Diet SBM ($p = 0.060$). Considering the higher amount of post-ruminally degraded $InsP_6$ for Diet RSM, it is likely that a certain proportion of the released MI from $InsP_6$ degradation in the lower digestive tract of wethers was absorbed and reached the bloodstream, contributing to a higher MI concentration in the blood plasma compared with Diet SBM. An association between ileal and blood MI concentrations was found in pigs and chicken (Sommerfeld et al. 2018; Rosenfelder-Kuon et al. 2020; Klein et al. 2021). To the best of our knowledge, studies on MI metabolism in ruminants have not yet been published. The degree to which blood MI is indicative of dietary $InsP_6$ degradation in ruminants requires further investigation. Notably, microorganisms in the digestive tract can both take up and synthesise MI (Reynolds 2009), which may interfere with the blood MI concentration.

5. Conclusion

The degradation of $InsP_6$ in wethers differed markedly when either RSM or SBM was included in the feed. The high content of $InsP_6$ in RSM resulted in a higher amount of $InsP_6$ degradation for Diet RSM compared to Diet SBM, whereas more easily degradable $InsP_6$ in SBM rendered the extent of ruminal and total tract $InsP_6$ disappearance for Diet SBM higher than that for Diet RSM. Compared with ruminal degradation, post-ruminal $InsP_6$ degradation was negligibly low. Along with the observed consistency between ruminal $InsP_6$ disappearance in the wethers and *in situ* calculated $InsP_6ED_2$, this suggests that $InsP_6$-P from feed available to animals is almost entirely derived from $InsP_6$ degradation in the rumen at a low rumen passage rate. Further research regarding phytase-producing microbiota may be helpful in understanding the effect of adaptation to $InsP_6$ degradation in different diets.

Acknowledgments

This study was supported by the Hohenheim University Foundation following a donation from alumni Dr Gerster. We would like to thank Heike Trapp and Lisa Uhland for their help in carrying out the experiments. We also appreciate the chemical analyses conducted by the laboratory team at the Animal Nutrition Department.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This project was funded by the Hohenheim University Foundation following a donation from alumni Dr Gerster, which is gratefully acknowledged.

ORCID

Yung-Ping Chi http://orcid.org/0000-0003-2117-6606
Eva Haese http://orcid.org/0000-0001-7795-1750
Markus Rodehutscord http://orcid.org/0000-0003-3156-7889

References

Bitar K, Reinhold JG. 1972. Phytase and alkaline phosphatase activities in intestinal mucosae of rat, chicken, calf, and man. Biochim Biophys Acta. 268:442–452.

Boguhn J, Baumgärtel T, Dieckmann A, Rodehutscord M. 2009. Determination of titanium dioxide supplements in different matrices using two methods involving photometer and inductively coupled plasma optical emission spectrometer measurements. Arch Anim Nutr. 63:337–342.

Brask-Pedersen DN, Glitsø LV, Skov LK, Lund P, Sehested J. 2011. Effect of exogenous phytase on feed inositol phosphate hydrolysis in an in vitro rumen fluid buffer system. J Dairy Sci. 94:951–959.

Brask-Pedersen DN, Glitsø LV, Skov LK, Lund P, Sehested J. 2013. Effect of exogenous phytase on degradation of inositol phosphate in dairy cows. J Dairy Sci. 96:1691–1700.

Castillo-González A, Burrola-Barraza M, Domínguez-Viveros J, Chávez-Martínez A. 2014. Rumen microorganisms and fermentation. Arch Med Vet. 46:349–361.

Eeckhout W, De Paepe M. 1994. Total phosphorus, phytate-phosphorus and phytase activity in plant feedstuffs. Anim Feed Sci Technol. 47:19–29.

GfE [Gesellschaft für Ernährungsphysiologie]. 2022. Recommended protocol for the determination of nutrient disappearance in situ for estimation of ruminal degradation. Proc Soc Nutr Physiol. 31:177–189.

Gillberg L, Törnell B. 1976. Preparation of rapeseed protein isolates. Dissolution and precipitation behavior of rapeseed proteins. J Food Sci. 41:1063–1069.

Gillespie J, Rogers SW, Deery M, Dupree P, Rogers JC. 2005. A unique family of proteins associated with internalized membranes in protein storage vacuoles of the Brassicaceae. Plant J. 41:429–441.

Haese E, Krieg J, Grubješić G, Feyder A, Rodehutscord M. 2020. Determination of in situ ruminal degradation of phytate phosphorus from single and compound feeds in dairy cows using chemical analysis and near-infrared spectroscopy. Animal. 14:1461–1471.

Haese E, Lengowski M, Gräter E, Föll A, Möhring J, Steingass H, Schollenberger M, Rodehutscord M. 2017b. Ruminal phytate degradation of maize grain and rapeseed meal in vitro and as affected by phytate content in donor animal diets and inorganic phosphorus in the buffer. J Anim Physiol Anim Nutr. 101:868–880.

Haese E, Möhring J, Steingass H, Schollenberger M, Rodehutscord M. 2017a. Effect of dietary mineral phosphorus and phytate on in situ ruminal phytate disappearance from different concentrates in dairy cows. J Dairy Sci. 100:3672–3684.

Haese E, Müller K, Steingass H, Schollenberger M, Rodehutscord M. 2014. Effects of mineral and rapeseed phosphorus supplementation on phytate degradation in dairy cows. Arch Anim Nutr. 68:478–491.

Haese E, Titze N, Rodehutscord M. 2022. In situ ruminal disappearance of crude protein and phytate from differently processed rapeseed meals in dairy cows. J Sci Food Agric. 102:2805–2812.

Huber K. 2016. Cellular myo-inositol metabolism. In: Walk C, Kühn I, Stein H, Kidd M Rodehutscord M, editors. Phytate destruction - consequences for precision animal nutrition. Wageningen, The Netherlands: Wageningen Academic Publishers; p. 53–60

Jarrett JP, Wilson JW, Ray PP, Knowlton KF. 2014. The effects of forage particle length and exogenous phytase inclusion on phosphorus digestion and absorption in lactating cows. J Dairy Sci. 97:411–418.

Kebreab E, Shah MA, Beever DE, Humphries DJ, Sutton JD, France J, Mueller-Harvey I. 2005. Effects of contrasting forage diets on phosphorus utilisation in lactating dairy cows. Livest Prod Sci. 93:125–135.

Kincaid RL, Garikipati DK, Nennich TD, Harrison JH. 2005. Effect of grain source and exogenous phytase on phosphorus digestibility in dairy cows. J Dairy Sci. 88:2893–2902.

Klein N, Papp M, Rosenfelder-Kuon P, Schroedter A, Avenhaus U, Rodehutscord M. 2021. Phosphorus digestibility and phytate degradation in pigs fed wheat-based diets with different intrinsic phytase activity and added microbial phytase. Arch Anim Nutr. 75:450–464.

Konishi C, Matsui T, Park W, Yano H, Yano F. 1999. Heat treatment of soybean meal and rapeseed meal suppresses rumen degradation of phytate phosphorus in sheep. Anim Feed Sci Technol. 80:115–122.

Liu KZ, Zhang YD, Yu ZT, Xu QB, Zheng N, Zhao SG, Huang GX, Wang JQ. 2021. Ruminal microbiota–host interaction and its effect on nutrient metabolism. Anim Nutr. 7:49–55.

Mambrini M, Peyraud JL. 1997. Retention time of feed particles and liquids in the stomaches and intestines of dairy cows. Direct measurement and calculations based on faecal collection. Reprod Nutr Dev. 37:427–442.

Mao S, Zhang M, Liu J, Zhu W. 2015. Characterising the bacterial microbiota across the gastrointestinal tracts of dairy cattle: membership and potential function. Sci Rep. 5:16116.

McDonald I. 1981. A revised model for the estimation of protein degradability in the rumen. J Agric Sci. 96:251–252.

Michell RH. 2008. Inositol derivatives: evolution and functions. Nat Rev Mol Cell Biol. 9:151–161.

Morse D, Head HH, Wilcox CJ. 1992. Disappearance of phosphorus in phytate from concentrates in vitro and from rations fed to lactating dairy cows. J Dairy Sci. 75:1979–1986.

Nelson TS, Daniels LB, Hall JR, Shields LG. 1976. Hydrolysis of natural phytate phosphorus in the digestive tract of calves. J Anim Sci. 42:1509–1512.

Ørskov ER, McDonald I. 1979. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. J Agric Sci. 92:499–503.

Park W-Y, Matsui T, Konishi C, Kim S-W, Yano F, Yano H. 1999. Formaldehyde treatment suppresses ruminal degradation of phytate in soyabean meal and rapeseed meal. Br J Nutr. 81:467–471.

Park W-Y, Matsui T, Yano H. 2002. Post-ruminal phytate degradation in sheep. Anim Feed Sci Technol. 101:55–60.

Raun A, Cheng E, Burroughs W. 1956. Ruminant nutrition, phytate phosphorus hydrolysis and availability to rumen microorganisms. J Agric Food Chem. 4:869–871.

Ray PP, Jarrett J, Knowlton KF. 2013. Effect of dietary phytate on phosphorus digestibility in dairy cows. J Dairy Sci. 96:1156–1163.
Ray PP, Shang C, Pearson RE, Knowlton KF. 2012. Disappearance of infused phytate from the large intestine of dairy heifers. J Dairy Sci. 95:5927–5935.
Reynolds TB. 2009. Strategies for acquiring the phospholipid metabolite inositol in pathogenic bacteria, fungi and protozoa: making it and taking it. Microbiology. 155:1386–1396.
Rodehutscord M, Sommerfeld V, Kühn I, Bedford MR. 2022. Phytases: potential and limits of phytate destruction in the digestive tract of pigs and poultry. In: Bedford M, Partridge G, Hruby M Walk C, editors. Enzymes in farm animal nutrition. 3rd ed. Oxfordshire, UK ; Boston, MA: CAB International; p. 124–152
Rosenfelder-Kuon P, Klein N, Zegowitz B, Schollenberger M, Kühn I, Thuringer L, Seifert J, Rodehutscord M. 2020. Phytate degradation cascade in pigs as affected by phytase supplementation and rapeseed cake inclusion in corn–soybean meal-based diets. J Anim Sci. 98:skaa053.
Seifried N, Steingass H, Hoffmann N, Rodehutscord M. 2017. In situ starch and crude protein degradation in the rumen and in vitro gas production kinetics of wheat genotypes. J Anim Physiol Anim Nutr. 101:779–790.
Sommerfeld V, Künzel S, Schollenberger M, Kühn I, Rodehutscord M. 2018. Influence of phytase or myo-inositol supplements on performance and phytate degradation products in the crop, ileum, and blood of broiler chickens. Poult Sci. 97:920–929.
Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten (VDLUFA). 2012. Handbuch der Landwirtschaftlichen Versuchs- und Untersuchungsmethodik (VDLUFA-Methodenbuch), Bd. III: Die chemische Untersuchung von Futtermitteln. Darmstadt, Germany: VDLUFA-Verlag.
Wang L, Shah AM, Liu Y, Jin L, Wang Z, Xue B, Peng Q. 2020. Relationship between true digestibility of dietary phosphorus and gastrointestinal bacteria of goats. PLoS One. 15: e0225018.
Wulf M, Südekum K-H. 2005. Effects of chemically treated soybeans and expeller rapeseed meal on in vivo and in situ crude fat and crude protein disappearance from the rumen. Anim Feed Sci Technol. 118:215–227.
Yanke LJ, Bae HD, Selinger LB, Cheng KJ. 1998. Phytase activity of anaerobic ruminal bacteria. Microbiology. 144:1565–1573.
Zeller E, Schollenberger M, Kühn I, Rodehutscord M. 2015. Hydrolysis of phytate and formation of inositol phosphate isomers without or with supplemented phytases in different segments of the digestive tract of broilers. J Nutr Sci. 4:e1.

4.2 MANUSCRIPT 2

Linkage of *in situ* ruminal crude protein degradation with ruminal degradation of amino acids and phytate from different soybean meals in dairy cows

Natascha Titze[1], Yung-Ping Chi[1], Eva Haese[2], Jens Hartung[3], and Markus Rodehutscord[1]

[1]Institute of Animal Science, University of Hohenheim, Stuttgart, Germany

[2]Institute of Animal Science, University of Bonn, Bonn, Germany

[3]Institute of Crop Science, University of Hohenheim, Stuttgart, Germany

Published in the Journal of Dairy Science (2023), 107: 2011–2025

The original publication is available at

https://doi.org/10.3168/jds.2023-23587

DOI: 10.3168/jds.2023-23587

J. Dairy Sci. 107:2011–2025
https://doi.org/10.3168/jds.2023-23587

Linkage of in situ ruminal degradation of crude protein with ruminal degradation of amino acids and phytate from different soybean meals in dairy cows

N. Titze,[1]* Y.-P. Chi,[1] E. Haese,[1]† J. Hartung,[2] and M. Rodehutscord[1]
[1]Institut für Nutztierwissenschaften, Universität Hohenheim, 70599 Stuttgart, Germany
[2]Institut für Kulturpflanzenwissenschaften, Universität Hohenheim, 70599 Stuttgart, Germany

ABSTRACT

The objectives of this study were to determine the range in ruminal degradability of crude protein (CP) and intestinal digestibility of rumen undegradable protein in commercial soybean meal (SBM) and to investigate the range in in situ ruminal AA and phytate ($InsP_6$) degradation and their relationship to CP degradation. An in situ study was conducted using 3 lactating Jersey cows with permanent rumen cannulas. Seventeen SBM variants from Europe, Brazil, Argentina, North America, and India were tested for ruminal CP and AA degradation, and in vitro intestinal digestibility of rumen undegradable protein. Nine variants were used to investigate the ruminal degradation of $InsP_6$. The estimated rapidly degradable fraction (a) of CP showed an average value of 4.5% (range: 0.0%–9.0%), the slowly degradable fraction (b) averaged 95% (91%–100%), and the potential degradation was complete for all 17 SBM variants. The degradation of fraction b started after a mean lag phase of 1.7 h (1.1–2.0 h) at an average rate (c) of 10% per hour, but with a high range from 4.5% to 14% per hour. Differences in the degradation parameters induced a considerable range in CP effective degradation at a rumen passage rate of 6% per hour ($CPED_6$) from 38% to 67%; hence, the concentration of rumen undegradable protein varied widely from 33% to 62%. The range in AA degradation between the SBM variants was high, with Ser showing the widest range, from 28% to 96%, and similar for the other AA. The regression equations showed close relationships between CP and AA degradation after 16 h of in situ incubation. However, the slopes of the linear regressions were significantly different between AA, suggesting that degradation among individual AA differs upon a change in CP degradation. The concentrations of $InsP_6$ and myo-inositol pentakisphosphate in bag residues in the in situ study decreased constantly with longer ruminal incubation times. The ruminal degradation parameters of $InsP_6$ ranged from 11% to 37% for fraction a, 63% to 89% for fraction b, and from 7.7% to 21% per hour for degradation rate c, with average values of 21%, 79%, and 16% per hour, respectively. The calculated $InsP_6$ effective degradation at a rumen passage rate of 6% per hour ($InsP_6ED_6$) varied from 61% to 84% among the SBM variants. Significant correlations were detected between $InsP_6ED_6$ and $CPED_6$ and between $InsP_6ED_6$ and chemical protein fractions A, B1, B2, B3, and C. Linear regression equations were developed to predict ruminal $InsP_6$ degradation using $CPED_6$ and chemical protein fractions B3 and C chosen by a stepwise selection procedure. We concluded that a high range in CP, AA, and $InsP_6$ degradation exists among commercial SBM, suggesting that general degradability values may not be precise enough for diet formulation for dairy cows. Degradation of CP in SBM may be used to predict rumen degradation of AA and $InsP_6$ using linear regression equations. Degradation of CP and $InsP_6$ could also be predicted from the chemical protein fractions.

Key words: chemical protein fractions, CNCPS, rumen, prediction equations

INTRODUCTION

Intensive milk production systems rely on diets with high nutrient density, including a large proportion of protein feed, to meet the requirements of dairy cows. An important goal in diet formulation for lactating dairy cows is to maximize microbial protein synthesis in the rumen and meet the demands of RUP and EAA for optimal milk production without wasting dietary protein, thereby improving N efficiency. For this purpose, the feeding value of these protein feeds and its range must be known or predicted with confidence. We know that different feedstuffs vary widely in ruminal protein degradation, intestinal digestibility, and AA composition of RUP (NASEM, 2021). Such differences

Received April 6, 2023.
Accepted September 20, 2023.
*Corresponding author: inst450@uni-hohenheim.de
†Current address: Institute of Animal Science, University of Bonn, 53115 Bonn, Germany.

also exist within one feedstuff, and studies have shown a wide range in the rumen degradation behavior of CP and AA in rapeseed meal (**RSM**; Steingass et al., 2013), dried distillers grains with solubles (**DDGS**; Westreicher-Kristen et al., 2013), lupin grains (Titze et al., 2019), and pea grains (Titze et al., 2021).

Although soybean meal (**SBM**) is one of the most common protein feeds in the diets of lactating dairy cows, studies on the range in RUP in SBM from different processing plants are scarce. Some studies have evaluated ruminal CP and AA degradation of SBM and the influence of an additional chemical or thermal treatment (Harstad and Prestløkken, 2000; Awawdeh et al., 2007; Borucki Castro et al., 2007) or compared SBM to other protein feedstuffs (Mjoun et al., 2010; Maxin et al., 2013). However, no study has examined the range in the feeding value of standard solvent-extracted SBM of different origins without additional chemical or physical treatment using an extensive sample set.

Moreover, substantial progress has been gained in the understanding of AA nutrition and metabolism in ruminants over the past few years (Schwab and Broderick, 2017), and consideration of the degradation of individual AA to refine the N supply to animals is important. However, it has been suggested that prediction of the AA composition of RUP from the AA profile of the feed protein is not possible (Cozzi et al., 1995), whereas Steingass et al. (2013) and Westreicher-Kristen et al. (2013) showed that ruminal CP and AA degradation are closely related in RSM and DDGS, and that degradation of individual AA can be accurately predicted from CP degradation. Therefore, we hypothesized that the prediction of AA degradation from CP degradation might also be possible for SBM.

A close relationship between CP and phytic acid (*myo*-inositol 1,2,3,4,5,6 hexakis dihydrogen phosphate, **$InsP_6$**) disappearance in RSM and SBM has been reported (Haese et al., 2017). Rapeseed and soybeans are relatively high in P concentration and most of the P is present in the salt form of $InsP_6$ (phytate) and is located in a protein-rich structure. Although $InsP_6$ degradation has been found to vary in a pattern similar to that of CP degradation in RSM (Haese et al., 2022), the range in ruminal $InsP_6$ degradation from commercial SBM in relation to CP degradation has not yet been studied. Our second hypothesis was that $InsP_6$ degradation is related to CP degradation in SBM. The aims of this study were to (1) determine the range in the in situ ruminal degradation of CP and the intestinal digestibility of rumen undegradable protein (**ID_{RUP}**) for different SBM; (2) investigate the range of in situ ruminal AA and $InsP_6$ degradation and their relationship with CP degradation; and (3) predict CP and $InsP_6$ degradation from chemical protein fractions.

MATERIALS AND METHODS

Soybean Variants

Seventeen commercially available solvent-extracted SBM variants from Europe (n = 6), Brazil (n = 6), Argentina (n = 2), North America (n = 2), and India (n = 1) were tested for ruminal CP and AA degradation and in vitro ID_{RUP}. Nine samples (no. 2, 4, 5, 9, 10, 11, 12, 14, and 18) were chosen because they covered a wide range of CP degradability and were used to investigate ruminal degradation of $InsP_6$, including analysis of less phosphorylated inositol phosphates ($InsP_{3\text{-}5}$). The variants were part of a larger set from a companion study that investigated the AA digestibility of 18 SBM in cecectomized laying hens (Siegert et al., 2023), which also contains a detailed description of all chemical analyses of the sample material used in this study, as well as all analytical values for each variant, including DM, CP, crude fat, crude ash, crude fiber, NDF assayed with a heat-stable amylase and expressed exclusive of residual ash, ADF expressed exclusive of residual ash, neutral detergent insoluble nitrogen, acid detergent insoluble nitrogen, starch, sugar, gross energy, AA, tannins, protein dispersibility index, KOH solubility, trypsin inhibitor activity, urease activity, in vitro solubility, inositol phosphate isomers, and particle size distribution. The numbering of the variants used in this study is the same as that used by Siegert et al. (2023). However, for one variant (no. 3) not enough material was left from the study by Siegert et al. (2023), which explains why we investigated only 17 variants. For the 17 SBM under study, concentrations (g/kg of DM) of CP and of the first limiting AA for milk production, Met, Lys, and His, ranged from 498 to 564, 7.2 to 8.9, 30.7 to 39.7, and 14.1 to 18.2, respectively (Table 1). The average $InsP_6$ concentration of the 9 SBM variants was 14.0 g/kg of DM (12.3–16.8 g/kg of DM) and average $InsP_5$ concentration was 2.6 g/kg of DM (1.9–4.0 g/kg of DM). Traces of $InsP_4$ were detected (up to 0.6 g/kg of DM) in the SBM, but $InsP_3$ was not detectable. Protein fractionation was based on the method described by Licitra et al. (1996) and analyzed as described by Titze et al. (2019). Protein fractions were calculated according to the Cornell Net Carbohydrate and Protein System (**CNCPS**; Sniffen et al., 1992) and ranged (percent of CP) between 0.0 and 5.2, 0.3 and 20.3, 71.1 and 85.8, 3.0 and 20.8, and 0.8 and 1.8 for fractions A, B1, B2, B3, and C, respectively (Table 1; Supplemen-

Table 1. Concentration of analyzed nutrients (g/kg of DM) and chemical protein fractions (% of CP) in soybean meals (n = 17 variants)[1]

	17 soybean meal variants				
Item	Mean	SD	Minimum	Maximum	CV (%)
DM	890	3.90	883	896	0.44
CP	527	21.9	498	564	4.16
Crude fat	22.8	4.49	15.0	32.0	19.7
Crude ash	76.8	7.27	70.0	100	9.47
aNDFom	43.8	6.63	30.0	54.0	15.2
ADFom	160	33.94	110	232	21.2
Starch	57.7	7.83	46.0	70.0	13.6
AA					
Ala	24.3	1.33	22.8	28.5	5.45
Arg	38.5	2.70	36.3	46.9	7.00
Asx[2]	63.8	4.18	58.6	76.0	6.55
Cys	7.33	0.38	6.60	8.20	5.12
Glx[2]	103	6.83	91.8	118	6.64
Gly	23.1	1.30	21.8	27.2	5.60
His	15.5	0.92	14.1	18.2	5.94
Ile	26.4	1.92	23.6	30.3	7.27
Leu	43.1	2.73	39.9	51.0	6.33
Lys	33.4	2.09	30.7	39.7	6.25
Met	7.85	0.41	7.20	8.90	5.28
Phe	29.9	2.55	26.2	34.5	8.52
Pro	27.3	1.87	25.4	32.7	6.83
Ser	27.7	2.05	25.9	33.9	7.38
Thr	22.0	1.30	20.1	25.3	5.89
Tyr	18.4	1.21	17.0	21.9	6.55
Val	25.8	1.63	23.8	30.7	6.30
Chemical protein fraction[3]					
A	1.52	1.92	0.00	5.20	126
B1	9.50	5.45	0.30	20.3	57.3
B2	79.8	3.77	71.1	85.8	4.72
B3	8.00	4.53	3.00	20.8	56.6
C	1.18	0.26	0.80	1.80	22.2
Inositol phosphate[4] (g/kg of DM)					
$InsP_6$	14.0	1.42	12.3	16.8	10.1
$InsP_5$	2.60	0.80	1.90	4.00	30.7
$InsP_4$	LOQ	—	—	—	—

[1]The values for the individual soybean meal variants are presented in Siegert et al. (2023) and Supplemental Table S1 (https://doi.org/10.5281/zenodo.7804535, Titze et al., 2023). aNDFom = NDF assayed with a heat-stable amylase and expressed exclusive of residual ash; ADFom = ADF expressed exclusive of residual ash.

[2]Asp and Asn, and Glu and Gln, respectively, were detected together because the Asn and Gln side groups were lost during acid hydrolysis (Fontaine, 2003).

[3]A = NPN; B1 = true protein rapidly degradable in the rumen; B2 = true protein with intermediate degradation rate in the rumen; B3 = true protein with slow degradation rate in the rumen; C = unavailable or cell wall-bound true protein.

[4]Concentrations of inositol phosphates, including phytate ($InsP_6$) and the sum of less phosphorylated inositol phosphate isomers ($InsP_5$ and $InsP_4$), are summarized for the 9 samples under study (no. 2, 4, 5, 9, 10, 11, 12, 14, 18); LOQ = below limit of quantification.

tal Table S1, https://doi.org/10.5281/zenodo.7804535; Titze et al., 2023).

Animals and Experimental Design

The experiment was conducted in accordance with German animal welfare legislation and approved by the Regierungspräsidium, Stuttgart, Germany (approval code V352/18 TE). Three lactating Jersey cows with an average BW of 470 kg (SD: 70 kg) and fitted with rumen cannulas were used for rumen in situ incubation. Cows were milked twice daily at 0500 and 1600 h and had a mean milk yield of 16 kg/d (SD: 0.5 kg/d) during the experimental period of 17 d until the incubation of all samples was finished. The cows were housed in a group in a freestall barn with cubicles covered with rubber mats and chopped straw and had free access to feed and water. During the experimental period cows consumed on average 16 kg/d of DM (SD: 3.4 kg/d) from a TMR that contained 25% corn silage, 25% grass silage, 17% grass hay, 16% mixed concentrate, 7% RSM, 6% toasted soybeans, 2% barley

straw, and 1% of a mineral mix on a DM basis. The diet contained (per kg of DM) 6.3 MJ NE_L, 133 g of CP, and 4.3 g of P.

In Situ Procedure and Determination of ID_{RUP}

The in situ procedure was based on the method proposed by Madsen and Hvelplund (1994) with minor modifications (Seifried et al., 2017). Samples (8 g of DM) were weighed into polyester bags (Ankom Co., Macedon, NY; pore size: 50 ± 3 µm; internal dimensions: 10 × 20 cm). After conditioning in warm tap water (~39°C) for 1 min, bags were inserted into the rumen for time spans of 2, 4, 6, 8, 12, 16, 24, 48, and 72 h. The incubation scheme was the same for all cows, meaning that bags of all variants for a specific time span were incubated at the same day and time in all 3 cows. However, variant and time span were at least replicated on 2 different days in all cows. After removal from the rumen, bags were rinsed with cold tap water and frozen. After the end of all in situ incubations, bags were thawed and washed in a washing machine (type WM14A160; Siemens GmbH, Munich, Germany) for 15 min without centrifugation, dried at 60°C for 24 h, and weighed. For the determination of the initial 0-h time point, 3 bags per SBM variant without ruminal preincubation were rinsed as described previously. Because leftovers in the bags decreased with longer ruminal incubation times and to obtain sufficient residue for chemical analysis for each variant at any incubation time, the number of bags per cow and time point differed; however, at least 9 measurements (3 bags × 3 cows) were performed at each time point. The residues per cow and incubation time were pooled and pulverized using a vibrating cup mill (Pulverisette; Fritsch GmbH, Idar-Oberstein, Germany) before further analysis. The water solubility of each SBM was determined over filter paper according to Madsen and Hvelplund (1994) and used to estimate small particle losses by subtraction from the initial 0 h loss.

To estimate ruminal degradation kinetics and effective degradation (**ED**), near-infrared spectroscopy (SpectraStar 2500X software: Unity InfoStar Version 3.11.1; Unity Scientific, Brookfield, CT) was used to determine the N concentration in all SBM samples and residues obtained from the bags. From a total of 425 samples in this study, a database of 333 samples, including SBM and their in situ bag residues (222 for calibration, 111 for validation) with N concentrations determined by chemical analysis (VDLUFA, 2007), was used for new calibration development according to the approach described by Krieg et al. (2018). Reference values ranged from 1.92% to 13.60% N on a DM basis. The best performance was achieved using a wavelength segment of 730 to 2,450 nm and a first derivative of the spectra. The calibration used had a standard error of calibration of 0.15% DM and a standard error of prediction of 0.21% DM, and the validation step showed an R^2 of 1.00 between the measured and predicted values. The slope and intercept of the validation set were 0.999 and 0.002, respectively, and not different from 1 and 0, respectively ($P < 0.05$). Crude protein was calculated as total N × 6.25.

Amino acids were determined in SBM and bag residues after incubation for 16 h. Determination on an L-8900 amino acid analyzer (VWR, Hitachi Ltd., Tokyo, Japan) followed sample oxidation and acid hydrolysis according to the protocol described by Rodehutscord et al. (2004). Residues of 16-h ruminal incubation were also used for in vitro determination of ID_{RUP} based on the 3-step method of Calsamiglia and Stern (1995) and measured as described by Grubješić et al. (2020).

Nine SBM variants and their corresponding bag residues were analyzed for $InsP_{3\ 6}$. Samples were extracted using the method described by Zeller et al. (2015), with slight modifications, as described by Sommerfeld et al. (2018). Briefly, 0.05 g of each sample was extracted with a solution of 0.2 *M* EDTA and 0.1 *M* sodium fluoride (pH = 8) under agitation for 30 min and was centrifuged at 12,000 × *g* for 15 min at 6°C. After centrifugation following 2 extractions, the supernatants were combined and 1 mL therefrom was centrifuged at 14,000 × *g* for 15 min at 4°C and filtered. After centrifugation again at 14,000 × *g* for 30 min at 4°C, the isomers of $InsP_{3\ 6}$ in the filtrates were measured using high-performance ion chromatography (ICS-3000 system, Dionex, Idstein, Germany).

Calculations and Statistical Analysis

Samples were created by pooling residues of all bags from cow *j*, SBM sample *i* at incubation time *t*. From these samples, degradation of CP, $InsP_6$, and AA (y) was calculated from the quantity of nutrients in the sample after incubation $\left(y_{bag\ residue_{ijt}}\right)$ and the quantity before the incubation started $\left(y_{feed_{ij}}\right)$ as

$$y_{ijt}\,(\%) = 100 - \frac{y_{bag\ residue_{ijt}}}{y_{feed_{ij}}} \cdot 100. \qquad [1]$$

The quantities of $y_{bag\ residue_{ijt}}$ and $y_{feed_{ij}}$ were calculated from the amount of DM in the bags pooled within the sample multiplied by the associated analyzed concentrations of CP, $InsP_6$, or a specific AA.

For each variant and cow an exponential model including lag time (*lag*) was used to fit the ruminal

degradation of CP and $InsP_6$ (Ørskov and McDonald, 1979; McDonald, 1981) as

$$Deg\,(t) = a + b\left(1 - e^{-c \cdot 0.01 \cdot (t - lag)}\right), \quad [2]$$

where $Deg\,(t)$ represents the degradation (%) of CP or $InsP_6$ at time t (h), a (%) is the rapidly degradable fraction, b (%) is the slowly degradable fraction over time, c (% per hour) is the degradation rate of b, and lag (h) is the duration until the beginning of the degradation of b.

The CP ED at a rumen passage rate of 6% per hour ($CPED_6$) and that of $InsP_6$ ($InsP_6ED_6$) were calculated using a ruminal outflow of $k = 6\%$ per hour and the following equation (Wulf and Südekum, 2005):

$$xED_6\,(\%) = a + \frac{(b \cdot c)}{(c + k)} \cdot e^{-k \cdot 0.01 \cdot lag}, \quad [3]$$

and RUP_6 was calculated as

$$RUP_6\,(\%) = 100 - CPED_6. \quad [4]$$

For estimation of in vitro ID_{RUP} the calculation was as follows:

$$ID_{RUP}\,(\%) = \frac{N_{soluble}}{N_{incubated}} \cdot 100, \quad [5]$$

where $N_{soluble}$ is the quantity of soluble N determined in vitro (mg) and $N_{incubated}$ is the total N incubated with pepsin and pancreatin (mg).

Model parameters for the in situ data were estimated using an iterative least-squares procedure in GraphPad Prism (version 5.00, GraphPad Software Inc., San Diego, CA).

All other statistical analyses were performed using SAS software (SAS System for Windows, version 9.4, SAS Institute Inc., Cary, NC). To evaluate whether the variability of the degradation parameters and $CPED_6$, $InsP_6ED_6$, and AA degradation after 16 h was equal to zero, a chi-squared test was applied to test the null hypothesis H_0: $\sigma^2 \leq 0.01$ over the variance of a normally distributed population. The null hypothesis could be rejected for all corresponding measures. Therefore, the degradation parameters and $CPED_6$, $InsP_6ED_6$, and AA degradation after 16 h between SBM variants were compared using a one-factorial approach with the MIXED procedure using the following model:

$$Y_{ij} = \mu + SBM_i + A_j + e_{ij}, \quad [6]$$

where Y_{ij} is the observed degradation in the sample of cow j and SBM sample i, μ is the intercept, SBM_i is the fixed effect of the SBM sample i (i = 1–17 or 1–9), A_j is the random effect of animal (j = 1, 2, 3), and e_{ij} is the residual error of Y_{ij}. In the case of a significant global F-test for differences in SBM levels, individual differences between the SBM means were determined using Tukey's honestly significantly different test. To determine the correlation, PROC CORR was used, and linear regression equations for $InsP_6ED_6$ were tested using stepwise selection in the REG procedure.

For comparison of individual AA degradation and prediction of individual AA degradation from CP degradation, data from the 17 SBM variants and 3 cows were also used. Two models were fitted. First, the model from Equation 6 was fitted to each AA to estimate the mean degradation. Note that residuals showed heterogeneous variances as expected for proportions. Therefore, data were logit-transformed before analysis. To compare degradations from 2 AA, a bivariate model (Equation 7) was fitted to each pair of AA. The model can be described as

$$Y_{ijk} = \mu_k + SBM_{ik} + A_{jk} + e_{ijk}, \quad [7]$$

where k is the index of AA. Therefore, the 2 parameters μ_k represent the 2 AA degradations from the pair of AA considered. For the random effects and the error effects, unstructured 2 × 2 variance–covariance matrices were fitted with AA-specific variances on the diagonal and a covariance on the off-diagonal. The bivariate analysis accounts for the correlations due to analyzing measured traits from the same pooled bag sample. Again, observations were logit-transformed before analysis. Means from Equation 6 and mean comparisons from Equation 7 were used to perform a Fisher's least significant difference test. Results were presented via letter display (Piepho 2012). Finally, means were back-transformed for presentation purposes only. Standard errors were back-transformed using the delta method. Note that AA degradation was estimated alternatively using the method of White et al. (2017). They proposed to predict AA degradation by the ratio of undegraded total AA (TAA_{ij}) and undegraded CP (CP_{ij}) using the following equation:

$$Y'_{ij} = 100 - \frac{100 - Y_{ij}}{100 - TAA_{ij}} \times \left(100 - CP_{ij}\right). \quad [8]$$

For the ratio of undegradability of individual AA (**uAAi**) to total AA (**TAA**), data were logarithmically transformed. Both fractions were proportions that can be binomially distributed. According to Katz et

al. (1978), the ratio of 2 binomial distributed variables is approximately normally distributed. Therefore, the models from Equations 6 and 7 were applied to logarithmically transformed data, and no deviations from the assumed normality were found when checking residual plots. As before, means and mean differences were used to create a letter display. Afterward, means and their standard error were back-transformed for presentation purposes only.

To predict individual AA degradation from CP degradation, the models from Equations 6 and 7 were extended by fitting average CP across cows as a covariable. The models can be described as follows:

$$Y_{ij} = \mu + SBM_i + A_j + \beta_1 CP_{mean_i} + e_{ij}, \quad [9]$$

$$Y_{ijk} = \mu_k + SBM_{ik} + A_{jk} + \beta_{1k} CP_{mean_i} + e_{ijk}, \quad [10]$$

where CP_{mean_i} is the CP degradations of SBM variant i averaged across cows, β_1 and β_{1k} are the general or AA specific slope parameters. All other terms were analogously defined as in Equations 6 and 7, including the use of index k for the AA used. Note that β_1 was reported throughout the paper. Again, 2 × 2 variance–covariance matrices for all random effects and the error were assumed. Unfortunately, estimated variance parameters for animal effects were generally small, which resulted in convergence problems, if one or both variances were bounded at zero in the final REML iteration. To get convergence in all bivariate models, the covariance between animal effects was dropped from the model. Statistical significance was set at $P \leq 0.05$ in all cases.

For all analyses aiming at predicting AA degradation, a cross validation was added to evaluate how well the model may fit in the future. Therefore, data were split into 17 subsamples, each from a single SBM variant. Sixteen subsamples were used to estimate the model and predict data from the remaining subsamples. The cross validation can be considered as leave one SBM variant out cross validation. Data from the cross validation were used to estimate the coefficient of variation. Additionally, the root mean square error (**RMSE**) between predicted and observed AA degradation was estimated for both analyses of AA degradation.

RESULTS AND DISCUSSION

This study aimed to characterize ruminal CP, AA, and $InsP_6$ degradation, as well as ID_{RUP} for different commercial solvent-extracted SBM. Furthermore, the relationship between CP degradation, AA degradation, and $InsP_6$ degradation was determined, and the prediction of CP and $InsP_6$ degradation from the chemical protein fractions was investigated.

CP and AA

Water-soluble CP corrected for small particle losses in the in situ study was, on average, 3.6% and was only slightly lower than the uncorrected rapidly degradable fraction (a), which showed an average value of 4.5% (Table 2). For this reason, and because 6 out of 17 samples (no. 6, 11, 12, 15, 16, and 18) had a lower rapidly degradable fraction than water-soluble fraction (Supplemental Table S2, https://doi.org/10.5281/zenodo.7804535, Titze et al., 2023), no correction for small particles was applied, as only minor effects on kinetic parameters and CPED were observed (values not shown). This is consistent with the results of Benchaar et al. (2021), who found differences of only 1.5 percentage points for the CPED of SBM with or without correction for small particles, as fraction a in their study was only reduced from 27.7% to 24.4%. This indicates that although measurement of the water-soluble fraction should always be performed, correction of in situ CP degradability values for small particle losses is not generally advisable for every class of feedstuff.

Common SBM is characterized by a well-balanced EAA pattern similar to that of ruminal bacteria and rich in Lys with a slight deficiency in Met (Miranda et al., 2019). However, because of the extensive ruminal degradation generally described in feed libraries (67% for SBM; NASEM, 2021), the utilization of SBM by ruminants as a source of RUP and metabolizable EAA from RUP is limited. In this study, the slowly degradable fraction (b) averaged 95%, and the estimated maximum degradation ($a + b$) was complete for all samples. This is consistent with previous studies showing that almost the entirety of CP in SBM is potentially degradable by rumen microorganisms if no further chemical or physical treatment is applied (Cozzi et al., 1995; Borucki Castro et al., 2007; Benchaar et al., 2021). The degradation of fraction b started after a mean lag phase of 1.7 h (range: 1.1–2.0 h) at an average rate of 10% per hour, but with a high range of the c value from 4.5% to 14% per hour. Differences in degradation parameters induced a considerable range in $CPED_6$ with values from 38% to 67%; hence, the concentration of RUP_6 varied widely from 33% to 62% for the 17 SBM under study. Although the degradation parameters, CPED, and RUP were determined using a defined approach in the present study, they were within the range of published values for solvent-extracted SBM among different studies, or partly higher (Cozzi et al., 1995; Awawdeh et al.,

Table 2. Water solubility and estimated in situ degradation parameters of CP, $CPED_6$, RUP_6[1] (% of CP, unless otherwise stated), and intestinal digestibility of RUP (ID_{RUP})[2] of soybean meal (n = 17 variants)[3]

	17 soybean meal variants				
Item	Mean	SD	Minimum	Maximum	CV (%)
Water solubility	3.6	1.1	2.1	6.0	32
a	4.5	2.8	0.0	9.0	62
b	95	2.9	91	100	3.0
Lag (h)	1.7	0.3	1.1	2.0	16
c (% per h)	10	2.6	4.5	14	26
$CPED_6$	58	7.7	38	67	13
RUP_6	42	7.7	33	62	18
ID_{RUP}	93	2.2	87	96	2.4

[1]Water solubility determined with filter paper; a = rapidly degradable fraction; b = slowly degradable fraction; c = degradation rate; $CPED_6$ = calculated effective degradation of CP at a rumen passage rate of 6% per hour; RUP_6 = calculated rumen undegradable CP at a rumen passage rate of k = 6% per hour.

[2]ID_{RUP} = intestinal digestibility of RUP after 16 h of incubation in the rumen.

[3]The values for the individual soybean meal variants are presented in Supplemental Table S2 (https://doi.org/10.5281/zenodo.7804535, Titze et al., 2023).

2007; Benchaar et al., 2021). For instance, Harstad and Prestløkken (2000) and Benchaar et al. (2021) observed degradation rates of 5.9% and 11.5% per hour and a CPED of 52.2% or 66.2%, respectively. It cannot be ruled out whether discrepancies in published values of CP degradation for standard SBM are due to the sample material or if methodological alterations in the study design may have induced such differences among experiments, as small modifications in every step of the in situ procedure can affect the results of rumen degradation measurements (GfE, 2022). However, we incubated all samples using the same standardized procedure, and the influences of the study design on the ranges in ruminal degradation kinetics and ED were therefore unlikely or at least negligible.

The large differences in CP degradation may be a function of the soybean genotypes used and the processing conditions, which include the intensity of moisture and heat treatment. Faldet et al. (1992) found that RUP content in SBM increased at higher temperatures (120–160°C) and heating durations (10–120 min). To the best of our knowledge, the influence of soybean genotype on the ruminal CP degradability of SBM has not yet been studied. However, the composition of genotypes in SBM might differ considerably because the sample material came from different regions with varying vegetation lengths, determining which soybean variety could be grown. Bachtiar et al. (2022) found differences in in vitro DM digestibility among 30 different soybean genotypes from Indonesia, and Ayasan et al. (2019) detected different gas production rates for 5 genotypes from Turkey. Differences in the feeding value may be mainly due to ranges in the chemical composition. In our study CP degradation was significantly correlated with the chemical protein fractions, which will be discussed in more detail in a subsequent section. Nevertheless, the causes of the differences in the amount of RUP, although not fully understood, could also have led to differences in intestinal digestibility among the SBM variants. However, the ID_{RUP} was high for all SBM and ranged between 87% and 96%, with an average value of 93% (Table 2), which is similar to the value of 91% for solvent-extracted SBM presented by NASEM (2021). The overall high values of ID_{RUP} are consistent with previous results. Awawdeh et al. (2007) observed an average ID_{RUP} of 82% using the same in vitro procedure as in our study, and Harstad and Prestløkken (2000) found intestinal indigestibility of RUP measured with the mobile nylon bag technique to be 1.4% and 1.6% for differently processed SBM products without significant differences between samples.

With sufficient knowledge, rations can be balanced for individual AA, thereby driving the implementation of low-protein diets for economic and ecological reasons. White et al. (2017) argued that incorporating AA degradability into nutrition supply-requirement models is challenging because even an extensive literature search yields an incomplete database for important feeds. Until now, it has not been possible to consider the degradability values for individual AA in any feed-evaluation system, and it was deemed that a broader database would be needed before the differential profile of the RUP fraction of the feedstuff could be predicted with confidence (NASEM, 2021). Liebe et al. (2018) suggested that values on AA degradation should also include ruminal degradation of TAA, and White et al. (2017) proposed an approach of normalizing uAAi as a proportion of TAA to allow better integration of AA degradability values into feed libraries based on RUP. We characterized a wide range of solvent-extracted

SBM for AA degradability, and degradability of TAA showed a least squares mean (**LSM**) of 83%, whereas the LSM of individual AA differed between 80% for Ser and 84% for Glx (Table 3). Differences in degradation between individual AA were partly significant, as well as in cases when uAAi was expressed as a proportion of TAA (Table 3). The average ratio of uAAi:TAA was highest for Ser (1.12) and higher than 1.0 for Ala, Gly, Leu, Ser, Thr, and Tyr for all SBM. For Met (n = 16), Val (n = 15), Cys (n = 13), and Asx (n = 13) the majority of samples also showed higher undegradability of these AA than their corresponding TAA (Table 3 and Supplemental Table S3, https://doi.org/10.5281/zenodo.7804535, Titze et al., 2023). In contrast, Glx showed the lowest undegradability ratio (0.90), Arg and Lys also tended to be lower than 1.0 in all 17 SBM, and 15 out of 17 SBM showed lower undegradability for His compared with TAA. The average ratio of undegradability for Ile, Phe, and Pro tended to be 1.0. These results were in accordance with the reviewed data set of White et al. (2017), who found that undegradability of His tends to be lower than that of TAA, whereas undegradability of Arg and Lys depended on the feed category. Susmel et al. (1989) and Harstad and Prestløkken (2000) found the highest ED for Arg, Glu, and Lys in SBM. Differences among individual AA have also been reported by other authors, and Lys is often considered the most degradable AA (O'Mara et al., 1997; Borucki Castro et al., 2007). Glutamic acid was reported to be more degradable than the other AA in SBM products by O'Mara et al. (1997), and in the study of Cozzi et al. (1995) Arg, His, and Lys were the only AA with consistently lower concentrations after ruminal exposure for 0, 8, 12, 16, and 24 h compared with the original profile. In contrast, reports on the least degradable AA in SBM are more variable. Harstad and Prestløkken (2000) found that Ser, together with other AA, was less degradable than the sum of AA taken together. However, Met and the branched-chain AA were most often found to be the least degradable AA in SBM (Harstad and Prestløkken 2000; Borucki Castro et al., 2007; Maxin et al., 2013) and other feed categories (Erasmus et al., 1994; Mjoun et al., 2010; White et al., 2017). Generally, some studies indicated that EAA are degraded more slowly than non-EAA, which may be due to the different distribution of these AA in the feed proteins (Cozzi et al., 1995). Other authors suggested that ruminal bacteria use peptides containing hydrophilic AA faster than hydrophobic peptides (Griswold and Mackie 1997). However, results from other studies do not support these assumptions and showed that the degradation of individual AA is feed dependent (Susmel et al., 1989; Depardon et al., 1995). Therefore, more research on this topic is warranted as a targeted

Table 3. Adjusted medians and their SE[1] of in situ degradability of CP, total amino acids (TAA), and individual AA (AAi) after 16 h with corresponding undegradability ratios[2] of soybean meal (n = 17 variants)[3]

			AA																
Variable	CP	TAA	Ala	Arg	Asx[4]	Cys	Glx[4]	Gly	His	Ile	Leu	Lys	Met	Phe	Pro	Ser	Thr	Tyr	Val
Degradability (%)																			
Median	82$^{\text{eg}}$	83$^{\text{ae}}$	81$^{\text{ij}}$	83$^{\text{a}}$	82$^{\text{f}}$	82$^{\text{fgh}}$	84$^{\text{a}}$	80$^{\text{l}}$	82$^{\text{cd}}$	82$^{\text{cef}}$	81$^{\text{ij}}$	83$^{\text{ab}}$	81$^{\text{ij}}$	82$^{\text{bcef}}$	82$^{\text{de}}$	80$^{\text{k}}$	81$^{\text{ni}}$	81$^{\text{ij}}$	81$^{\text{i}}$
SE	9.9	8.6	11	9.6	10	9.8	9.7	10	9.6	9.9	11	9.1	10	10	9.6	11	10	11	10
Undegraded AAi/TAA																			
Median	—	—	1.07$^{\text{efl}}$	0.93$^{\text{jrtu}}$	1.01$^{\text{hrtus}}$	1.04$^{\text{ijk}}$	0.90$^{\text{lpsu}}$	1.10$^{\text{bcgj}}$	0.97$^{\text{qrou}}$	0.99$^{\text{imo}}$	1.07$^{\text{elf}}$	0.95$^{\text{t}}$	1.06$^{\text{ce}}$	0.98$^{\text{klnqr}}$	0.99$^{\text{eq}}$	1.12$^{\text{ab}}$	1.05$^{\text{efl}}$	1.07$^{\text{elrp}}$	1.06$^{\text{beh}}$
SE	—	—	0.01	0.01	0.00	0.01	0.01	0.02	0.01	0.01	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01

[a–u]Estimates with at least one identical letter within rows were not significantly different from each other ($P < 0.05$).

[1]For statistical comparison of the mean values of degradability and undegraded AAi/TAA standard error of the difference = 5.4 (range: 0.48–597) and 0.001 (range: 0.0004–0.004), respectively.

[2]Estimated ratios of undegraded AAi to TAA were calculated according to White et al. (2017).

[3]The values for the individual soybean meal variants are presented in Supplemental Table S3 (https://doi.org/10.5281/zenodo.7804535, Titze et al., 2023).

[4]Asp and Asn, and Glu and Gln, respectively, were detected together because the Asn and Gln side groups were lost during acid hydrolysis (Fontaine, 2003).

Table 4. Parameter estimates of the linear regression parameters and model fit criteria of AA degradation (y, %) from CP degradation (x, %) after 16 h of ruminal incubation (n = 17 soybean meal variants)

	17 soybean meal variants						
	Slope[1]		Intercept[2]		Accuracy of cross validation		
AA	Mean	SEM	Mean	SEM	R^2	CV	RMSE[3]
Ala	1.21[jmo]	0.04	−19.6	4.52	0.96	4.66	3.60
Arg	1.13[ck]	0.04	−11.2	3.96	0.96	4.00	3.18
Asx[4]	1.20[jn]	0.03	−18.5	4.28	0.96	4.45	3.47
Cys	1.09[nopqr]	0.03	−9.48	4.12	0.96	3.96	3.10
Glx[4]	1.17[hikl]	0.03	−13.9	4.70	0.95	4.92	3.94
Gly	1.17[f]	0.04	−17.1	4.35	0.96	4.48	3.45
His	1.16[e]	0.03	−13.8	4.00	0.96	4.21	3.32
Ile	1.15[j]	0.03	−13.6	5.10	0.95	5.04	3.96
Leu	1.22[blk]	0.05	−21.2	4.50	0.96	4.61	3.55
Lys	1.10[oq]	0.04	−9.04	4.10	0.96	4.13	3.29
Met	1.17[dip]	0.03	−16.4	4.33	0.96	4.27	3.31
Phe	1.21[bgh]	0.03	−18.3	5.52	0.94	5.62	4.42
Pro	1.11[bg]	0.06	−10.1	3.94	0.97	3.71	2.92
Ser	1.26[abc]	0.03	−25.4	4.41	0.97	4.31	3.28
Thr	1.21[adefg]	0.03	−19.9	4.86	0.95	4.96	3.84
Tyr	1.21[adeg]	0.04	−20.2	4.68	0.96	4.79	3.69
Val	1.13[almp]	0.04	−13.1	4.34	0.96	4.27	3.31

[a] [1]Estimates with at least one identical letter within a column were not significantly different from each other ($P < 0.05$).

[1]Slopes were all different from 1 ($P < 0.05$); SE of the difference for slope comparison = 0.008 (range: 0.0009 0.03).

[2]Intercepts were all different from 0 ($P < 0.05$).

[3]Root mean square error.

[4]Asp and Asn, and Glu and Gln, respectively, were detected together because the Asn and Gln side groups were lost during acid hydrolysis (Fontaine, 2003).

supplementation of rumen-protected AA instead of increasing several protein feeds in the diets may prevent diseases among animals and enhance their productivity while lowering environmental pollution (Kim and Lee 2021; Khan et al., 2023)

Compared with the differences between the average values of AA degradation within one SBM, the range in AA and CP degradation among the SBM variants was large. Among the meals studied, the degradability of the first limiting AA for milk production, Met, Lys, and His varied between 34% and 96%, 37% and 97%, and 34% and 97%, respectively. The other AA, whether essential or not, also showed high ranges (Supplemental Table S3).

The variable degradation of CP and AA observed in our study may indicate that the supplied amount of RUP and, hence, metabolizable EAA from RUP differ considerably among sources. Therefore, accurate information on ruminal degradability, instead of using average values for diet formulation, is of utmost importance to meet the animals' protein requirements and maximize animal performance while minimizing N losses.

Although the magnitude of the difference between CP and individual AA degradation was not large, it was significant in most cases, suggesting that the degradation of AA in SBM differs from that in CP (Table 3). The regression equations and accuracy of the cross validations showed close relationships between CP degradation and the degradation of Met and Lys (R^2 = 0.96; Table 4) and other AA showing R^2 values between 0.94 and 0.97 (Table 4). The slopes differed among the AA, suggesting that the degradation of each AA varied in magnitude with changes in CP degradation. Slopes and intercepts of the linear regression lines were all higher than 1 and 0, respectively. This can perhaps be explained by the part of CP that is nondegradable.

To the best of our knowledge, this is the first study to investigate the relationship between CP and AA degradation in SBM using the applied approach. Borucki Castro et al. (2007) tested 4 differently treated SBM products and found that within each product, the ED of individual AA was variable and not constant across the SBM. However, statistical validation of the first statement was missing in Borucki Castro et al. (2007), and 3 of their SBM products underwent additional treatment to enhance RUP, which may have altered AA degradation compared with standard solvent-extracted SBM. As previously discussed, other authors have reported differences in the degradation of individual

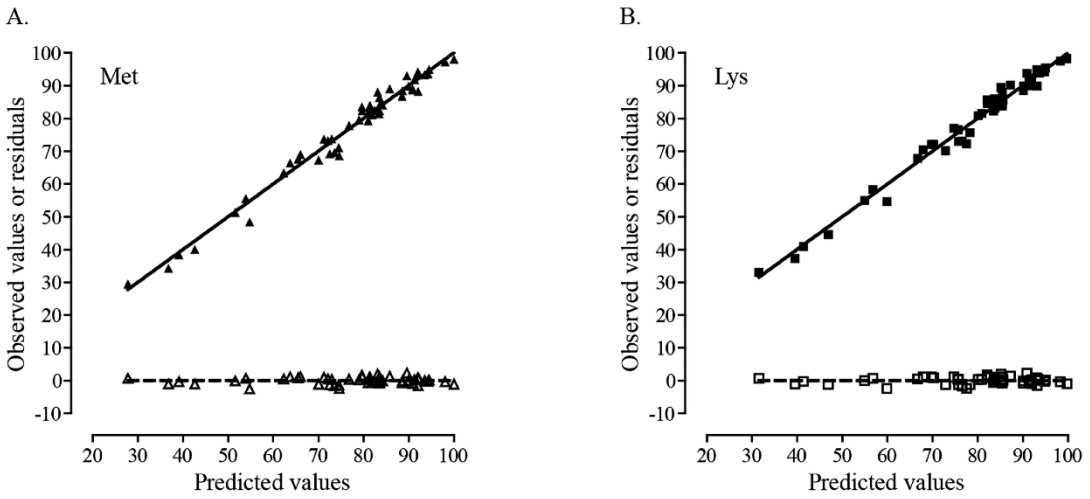

Figure 1. Diagnostic plots for predictions of ruminal degradation of Met (A) and Lys (B) from the degradation of CP after 16 h of ruminal incubation. Filled symbols and solid lines represent observed versus predicted data, and open symbols with broken lines represent studentized residuals versus predicted data. Intercepts of the regression lines are not different from 0, and slopes of the regression lines are not different from 1 (observed vs. predicted) and 0 (studentized residuals vs. predicted; $P > 0.99$; n = 51). The diagnostic plots for the other AA are presented in Supplemental Figure S1A–O (https://doi.org/10.5281/zenodo.7804535, Titze et al., 2023).

AA within the same meal. The application of a mean degradability value for all AA or the degradability of CP may lead to a biased calculation of the supply of EAA to the duodenum of cows. Linear regressions, as used in our study, can estimate the rumen degradation of all AA based on the CP degradation of SBM (Figure 1 and Supplemental Figure S1, https://doi.org/10.5281/zenodo.7804535, Titze et al., 2023). The diagnostic plots of the linear regression showed that the studentized residuals were not correlated with the prediction values. Slopes of studentized residuals regressed on the predicted degradation values for each AA were not significantly different from zero in any case. This implies that the error of the prediction did not depend on the AA degradation of the sample, indicating an unbiased model with which prediction of AA degradation is possible with similar accuracy over the entire range. Moreover, accuracy of cross validation to validate the model fit for future prediction showed overall high R^2 (0.94–0.97) and low RMSE (2.92–4.42; Table 4). This approach has previously been used to estimate AA degradation and, hence, the AA composition of the RUP of DDGS (Westreicher-Kristen et al., 2013) and RSM (Steingass et al., 2013). Hence, the linear regression approach for the prediction of AA degradation based on CP degradability appears to be applicable to different feeds. White et al. (2017) also showed a strong relationship between N (or CP) and TAA degradability over a wide range of feed categories and proposed that future feed libraries could include prediction of undegradability of EAA based on RUP content by multiplication with the ratios of EAA normalized to TAA. However, when AA degradability of the 17 SBM from this study was predicted from the ratio (uAAi/TAA) of each AA (Table 3) and their corresponding RUP after 16 h (values not shown), RMSE of the prediction model was 4.42% and therefore higher compared with the 2.40% of the regression approach, although cross validation of both approaches to evaluate how good the model may fit in the future showed similar RMSE, with 17.2% and 17.3% for the TAA and the linear regression approach, respectively. In conclusion, both approaches show that usage of CP degradability or undegradability measured by in situ procedure or possibly, in the future, estimated from laboratory measurements such as chemical protein fractions, could be a robust predictor for ruminal degradability of AA driving their implementation in feed evaluation systems.

Phytate

Overall, concentrations of $InsP_6$ and $InsP_5$ in the bag residues of the 9 SBM decreased with the incubation time from 0 h ($InsP_6$: 14.4 g/kg of DM; $InsP_5$: 2.5 g/kg of DM) to 24 h ($InsP_6$: 3.9 g/kg of DM; $InsP_5$: 0.7 g/kg of DM; Figure 2). After 48 h of ruminal incuba-

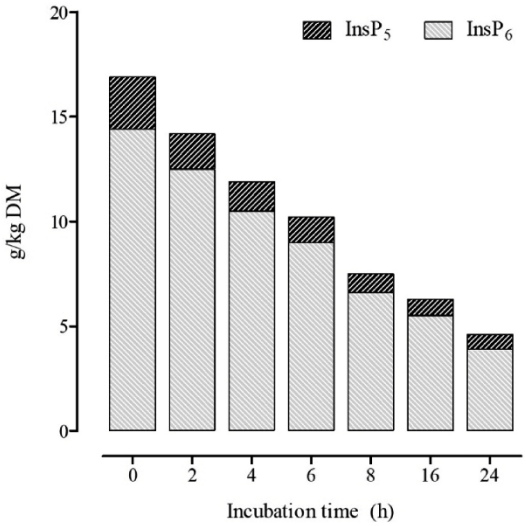

Figure 2. Mean concentrations of inositol phosphates including phytate ($InsP_6$) and the sum of less phosphorylated inositol phosphate isomers ($InsP_5$) in the bag residues of 9 soybean meal variants at different time periods of incubation in the rumen (g/kg of DM).

tion, the $InsP_6$ concentration was reduced to less than 1 g/kg of DM, $InsP_5$ was not detectable in most bag residues, $InsP_4$ was detected at very low concentration (0.1 g/kg of DM) until 2 h of ruminal incubation but was barely detectable from 4 h of incubation onwards, and $InsP_3$ was not detected in any bag residue. These changes over time are consistent with the results of previous in situ studies, showing that $InsP_6$ degradation in SBM begins soon after incubation and proceeds almost completely within 48 h without any accumulation of partially dephosphorylated InsP isomers (Haese et al., 2017). However, a wide range was observed in the in situ degradation parameters of $InsP_6$. The ruminal degradation parameters ranged from 11% to 37% for fraction *a*, 63% to 89% for fraction *b*, and 7.7% to 21% per hour for degradation rate *c* (Table 5) with average values of 21%, 79%, and 16% per hour, respectively. The calculated $InsP_6ED_6$ varied considerably among the SBM, ranging from 61% to 84%. The solubility and degradation rate of $InsP_6$ from treated oilseed meals are strongly affected by processing conditions such as toasting or desolventizing time (Haese et al., 2022), heating (Konishi et al., 1999; Wang et al., 2018), and formaldehyde treatment (Park et al., 1999). Such processing effects may at least partly explain the range in $InsP_6ED_6$ among the SBM in this study because different processing conditions in the oil plants can be assumed. The meals were obtained from the market, and no information on their origin, except the country, was available. Thus, intrinsic differences in the processed soybeans and agronomic conditions may also have contributed to the observed range.

Correlations and Relationship Between Degradation and Chemical Protein Fractions

Significant correlations were detected between the estimated parameters and ED of CP degradation with the chemical protein fractions. The lag phase of CP degradation was positively correlated with the chemical protein fraction B3 (r = 0.60). The CP degradation rate correlated with the chemical protein fractions B1 (r = 0.80), B3 (r = −0.88), and C (r = −0.56), and the same 3 fractions showed significant correlations with $CPED_6$ (B1: r = 0.76; B3: r = −0.90; C: r = −0.66). Differences in chemical protein fractions could be partly due to the range in soybean genotypes because protein composition varies among soybean varieties (Fehr et al., 2003; Pesic et al., 2005; Zilić et al., 2011). However, unpublished data from our department showed that the chemical protein fractions B1, B2, and B3 are especially influenced by thermal and hydrothermal treatments (sample material and processing conditions described in Kaewtapee et al., 2017), with B1 decreas-

Table 5. In situ degradation parameters and effective degradation of phytate ($InsP_6$; %, unless otherwise stated) for soybean meal (n = 9 variants, n = 3 replicates)[1]

	Variant no.									
Variable	2	4	5	9	10	11	12	14	18	SEM
a	37^{a}	26^{b}	23^{bc}	21^{c}	20^{c}	13^{d}	11^{d}	24^{bc}	13^{d}	1.62
b	63^{d}	72^{c}	77^{bc}	79^{b}	80^{b}	86^{a}	89^{a}	76^{bc}	87^{a}	1.86
c (%/h)	19^{a}	14^{b}	19^{a}	19^{a}	21^{a}	7.7^{d}	9.2^{cd}	21^{a}	12^{bc}	1.37
$InsP_6ED_6$	84^{a}	76^{c}	81^{b}	81^{b}	82^{ab}	61^{f}	65^{e}	83^{ab}	71^{d}	1.42

[a–f]Estimates with at least one identical letter within rows were not significantly different from each other ($P < 0.05$).

[1]*a* = rapidly degradable fraction; *b* = slowly degradable fraction; *c* = degradation rate; $InsP_6ED_6$ = calculated effective degradation of $InsP_6$ at a rumen passage rate of 6% per hour.

ing from 73% in raw soybean to less than 1% with increasing intensity of processing conditions, whereas B2 and B3 increased from 22% to 73% and 0% to 16%, respectively. Chemical protein fractions A and C were almost unaffected. These results are consistent with those of previous studies on the influence of microwave irradiation, electron beam irradiation, and roasting on the chemical protein fractions of whole soybeans (Akbarian et al., 2014; Golshan et al., 2019).

The application of dynamic models for precise diet formulation depends, in addition to other factors, largely on the estimates of feedstuffs feeding values. For example, CNCPS models CPED based on chemical protein fractions and defines the degradation rates for A, B1, B2, and B3 to calculate RUP values for a given passage rate (Sniffen et al., 1992). Using individual degradation rates for chemical protein fractions from CNCPS version 6.5 for the 17 SBM samples under study, the calculated $CPED_6$ values ranged between 62% and 67%, which is much less variable than in situ $CPED_6$ (Table 2), although a significant correlation was found ($r = 0.73$). Hence, in some cases, the RUP values for the SBM were considerably underestimated by this prediction model. Therefore, regression analysis was applied to estimate the in situ $CPED_6$ directly from chemical protein fractions without the use of fractional degradation rates. The following equation was developed based on stepwise selection using only significant variables, as $CPED_6$ (%) = 69.90 − 1.54 × B3, with B3 as percentage of CP ($R^2 = 0.82$; RMSE = 3.43).

When examining RSM, Haese et al. (2022) calculated significant correlations between $InsP_6ED$ and CPED and between $InsP_6ED$ and protein fractions B1, B2, and C. These relationships can be confirmed for the SBM of this study, where a significant correlation was observed between $InsP_6ED_6$ and $CPED_6$ (r = 0.88) and between $InsP_6ED_6$ and chemical protein fractions A, B1, B2, B3, and C (A: r = −0.91; B1: r = 0.69; B2: r = 0.70; B3: r = −0.85; C: r = −0.91). For SBM, $InsP_6$ was found to interact naturally with the protein in the seed (Prattley and Stanley, 1982; Hídvégi and Lásztity, 2002), which may be strengthened by the further formation of protein–phytate complexes during mechanical processing (Wang et al., 2018) and could explain the observed association between $InsP_6$ and CP degradation.

In contrast to the high digestibility of RUP from SBM in the intestine, the post-ruminal digestion of $InsP_6$ is very low (Chi et al., 2022), and degradation of $InsP_6$ in the large intestine does not vary with the flow of $InsP_6$ (Ray et al., 2012, 2013). Thus, increasing the amount of rumen undegraded $InsP_6$ does not increase P available for the animals but does increase excretion of $InsP_6$ in feces, which contradicts the requirement for high RUP content to support high rates of milk production and high milk quality.

Based on the observed range in the degradation of CP and $InsP_6$ and the significant correlation between them, we suggest equations to estimate ruminal $InsP_6$ degradation of commercial SBM depending on CP degradation values or CP fractionation when formulating diets, which has also been proposed for RSM (Haese et al., 2022). The linear regression equation for $InsP_6ED$ (y) depending on $CPED_6$ (x) was y (%) = 0.76x (%) + 34.7 ($R^2 = 0.77$; RMSE = 4.34; $P < 0.05$; Figure 3). For the linear regression based on chemical protein fractions, variables were selected as described above and resulted in $InsP_6ED_6$ (%) = 103 − 0.76·B3 − 15.4·C ($R^2 = 0.92$; RMSE = 2.80; $P < 0.05$). Although both approaches seem to be applicable, equations based on chemical protein fractions showed remarkably higher accuracy (higher R^2 and lower RMSE) than the regression equation based on CPED, which allows a more precise prediction of $InsP_6ED$ for individual SBM. Furthermore, an approach based on chemical protein fractions is less time-consuming, and the use of animals for in situ experiments is not necessary. To evaluate the applicability of one general equation for different feedstuffs, we also calculated $InsP_6ED$ for SBM in this study using the equations proposed for RSM by Haese et al. (2022),

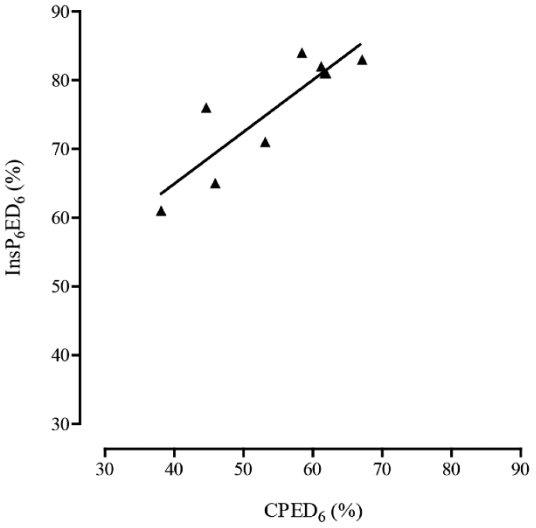

Figure 3. Linear regression for the calculated rumen effective degradation of phytate ($InsP_6ED$) depending on crude protein at a rumen passage rate of 6% per hour ($CPED_6$). Data (▲; $y = 0.76x + 34.7$; $R^2 = 0.77$; $P < 0.05$; root mean square error = 4.34; SE for intercept = 8.68; SE for slope = 0.16) are presented as the mean (n = 9 soybean meal variants).

which were $y = 1.01x - 2.38$ for $InsP_6ED$ (y) depending on CPED (x), and $InsP_6ED$ (%) = $-233.6 - 0.38 \cdot B1 + 6.45 \cdot B2 + 0.74 \cdot C$ at a rumen passage rate of 5% per hour. Nevertheless, the calculation based on equations developed for RSM resulted in a considerable underestimation and overestimation of $InsP_6ED$ by CPED and chemical protein fractions, respectively (values not shown), implying that the equations for one oilseed meal type may not be applicable to another. Oilseeds differ in their $InsP_6$:protein ratio (Kies et al., 2006), $InsP_6$ distribution (Prattley and Stanley, 1982; Gillespie et al., 2005), and occurrence of inorganic cations (Hídvégi and Lásztity, 2002) within the protein storage vacuole, which can influence the interaction between $InsP_6$ and CP and the degradation of $InsP_6$ and CP. In addition, storage proteins in seeds exhibit different chemical or physical features and reactions, such as the aggregation or denaturation of storage proteins during the production of oilseed meals, which can further affect the degradation of associated components (Yiu et al., 1983). Therefore, feedstuff-specific equations may be necessary to estimate the $InsP_6ED$.

CONCLUSIONS

Different SBM showed considerable range in ruminal degradation parameters and the ED of CP and $InsP_6$. Data on CP, AA, and $InsP_6$ degradability can be used to extend the databases for SBM as an important protein feed for dairy cows and to support the implementation of AA degradability values in protein evaluation systems. Close relationships between the CP degradation and degradation of individual AA and $InsP_6$ were detected, and regression equations were proposed and can be used to estimate the degradation values of CPED and $InsP_6ED$ of SBM based on chemical protein fractions.

ACKNOWLEDGMENTS

This study received no external funding. The contribution of master's student Thomas Liebske (University of Hohenheim, Stuttgart, Germany) for helping to carry out the in situ procedure is gratefully acknowledged. Supplemental material is available at https://doi.org/10.5281/zenodo.7804535 (Titze et al., 2023). The authors have not stated any conflicts of interest.

REFERENCES

Akbarian, A., M. Khorvash, G. R. Ghorbani, E. Ghasemi, M. Dehghan-Banadaky, P. Shawrang, and M. Hosseini Ghaffari. 2014. Effects of roasting and electron beam irradiating on protein characteristics, ruminal degradability and intestinal digestibility of soybean and the performance of dairy cows. Livest. Sci. 168:45–52. https://doi.org/10.1016/j.livsci.2014.07.019.

Awawdeh, M. S., E. C. Titgemeyer, J. S. Drouillard, R. S. Beyer, and J. E. Shirley. 2007. Ruminal degradability and lysine bioavailability of soybean meals and effects on performance of dairy cows. J. Dairy Sci. 90:4740–4753. https://doi.org/10.3168/jds.2007-0210.

Ayasan, T., M. Boga, M. Baylan, S. Ergul, H. Kutay, S. Naeim Saber, C. Mizrak, and P. Cubukcu. 2019. Determination of nutritive value of soybean varieties using in vitro methods and gas production technique. Iran. J. Appl. Anim. Sci. 9:603–608.

Bachtiar, T., M. Hanani, A. Anisiyah, W. Puspitasari, W. T. Sasongko, and T. Wahyono. 2022. Nutrient profile and in vitro digestibility of thirty Indonesian soybean genotypes grown at two different soil pH for selection as ruminant feed. Adv. Anim. Vet. Sci. 10:1818–1826. https://doi.org/10.17582/journal.aavs/2022/10.8.1818.1826.

Benchaar, C., F. Hassanat, K. A. Beauchemin, G. Gislon, and D. R. Ouellet. 2021. Diet supplementation with canola meal improves milk production, reduces enteric methane emissions, and shifts nitrogen excretion from urine to feces in dairy cows. J. Dairy Sci. 104:9645–9663. https://doi.org/10.3168/jds.2020-20053.

Borucki Castro, S. I., L. E. Phillip, H. Lapierre, P. W. Jardon, and R. Berthiaume. 2007. Ruminal degradability and intestinal digestibility of protein and amino acids in treated soybean meal products. J. Dairy Sci. 90:810–822. https://doi.org/10.3168/jds.S0022-0302(07)71565-5.

Calsamiglia, S., and M. D. Stern. 1995. A three-step in vitro procedure for estimating intestinal digestion of protein in ruminants. J. Anim. Sci. 73:1459–1465. https://doi.org/10.2527/1995.7351459x.

Chi, Y.-P., E. Haese, and M. Rodehutscord. 2022. Ruminal and postruminal phytate degradation of diets containing rapeseed meal or soybean meal. Arch. Anim. Nutr. 76:233–247. https://doi.org/10.1080/1745039X.2022.2164158.

Cozzi, G., I. Andrighetto, P. Berzaghi, and C. E. Polan. 1995. In situ ruminal disappearance of essential amino acids in protein feedstuffs. J. Dairy Sci. 78:161–171. https://doi.org/10.3168/jds.S0022-0302(95)76626-7.

Depardon, N., D. Debroas, and G. Blanchart. 1995. Breakdown of peptides from a soya protein hydrolysate by rumen bacteria. Simultaneous study of enzyme activities and of two physico-chemical parameters: Molecular weight and hydrophobicity. J. Sci. Food Agric. 68:25–31. https://doi.org/10.1002/jsfa.2740680105.

Erasmus, L. J., P. M. Botha, C. W. Cruywagen, and H. H. Meissner. 1994. Amino acid profile and intestinal digestibility in dairy cows of rumen-undegradable protein from various feedstuffs. J. Dairy Sci. 77:541–551. https://doi.org/10.3168/jds.S0022-0302(94)76982-4.

Faldet, M. A., L. D. Satter, and G. A. Broderick. 1992. Determining optimal heat treatment of soybeans by measuring available lysine chemically and biologically with rats to maximize protein utilization by ruminants. J. Nutr. 122:151–160. https://doi.org/10.1093/jn/122.1.151.

Fehr, W. R., J. A. Hoeck, S. L. Johnson, P. A. Murphy, J. D. Nott, G. I. Padilla, and G. A. Welke. 2003. Genotype and environment influence on protein components of soybean. Crop Sci. 43:511–514. https://doi.org/10.2135/cropsci2003.5110.

Fontaine, J. 2003. Amino acid analysis of feeds. Pages 15–40 in Amino Acids in Animal Nutrition. J. P. F. D'Mello, ed. CAB International, Wallingford, UK.

GfE. 2022. Recommended protocol for the determination of nutrient disappearance in situ for estimation of ruminal degradation. Proc. Soc. Nutr. Physiol. 31:177–189.

Gillespie, J., S. W. Rogers, M. Deery, P. Dupree, and J. C. Rogers. 2005. A unique family of proteins associated with internalized membranes in protein storage vacuoles of the Brassicaceae. Plant J. 41:429–441. https://doi.org/10.1111/j.1365-313X.2004.02303.x.

Golshan, S., R. Pirmohammadi, and H. Khalilvandi-Behroozyar. 2019. Microwave irradiation of whole soybeans in ruminant nutrition: Protein and carbohydrate metabolism in vitro and in situ. Vet. Res. Forum 10:343–350. https://doi.org/10.30466/vrf.2019.35896.

Griswold, K. E., and R. I. Mackie. 1997. Degradation of protein and utilization of the hydrolytic products by a predominant ruminal

bacterium, *Prevotella ruminicola* B1(4). J. Dairy Sci. 80:167–175. https://doi.org/10.3168/jds.S0022-0302(97)75924-1.

Grubješić, G., N. Titze, J. Krieg, and M. Rodehutscord. 2020. Ruminal fermentation characteristics and related feeding values of compound feeds and their constituting single feeds studied by using in vitro techniques. Animal 14:1829–1840. https://doi.org/10.1017/S1751731120000889.

Haese, E., J. Möhring, H. Steingass, M. Schollenberger, and M. Rodehutscord. 2017. Effect of dietary mineral phosphorus and phytate on in situ ruminal phytate disappearance from different concentrates in dairy cows. J. Dairy Sci. 100:3672–3684. https://doi.org/10.3168/jds.2016-11468.

Haese, E., N. Titze, and M. Rodehutscord. 2022. In situ ruminal disappearance of crude protein and phytate from differently processed rapeseed meals in dairy cows. J. Sci. Food Agric. 102:2805–2812. https://doi.org/10.1002/jsfa.11621.

Harstad, O., and E. Prestløkken. 2000. Effective rumen degradability and intestinal indigestibility of individual amino acids in solvent-extracted soybean meal (SBM) and xylose-treated SBM (SoyPass®) determined in situ. Anim. Feed Sci. Technol. 83:31–47. https://doi.org/10.1016/S0377-8401(99)00114-5.

Hídvégi, M., and R. Lásztity. 2002. Phytic acid content of cereals and legumes and interaction with proteins. Period. Polytech. Chem. Eng. 46:59–64.

Kaewtapee, C., M. Eklund, M. Wiltafsky, H.-P. Piepho, R. Mosenthin, and P. Rosenfelder. 2017. Influence of wet heating and autoclaving on chemical composition and standardized ileal crude protein and amino acid digestibility in full-fat soybeans for pigs. J. Anim. Sci. 95:779–788. https://doi.org/10.2527/jas.2016.0932.

Katz, D., J. Baptista, S. P. Azen, and M. C. Pike. 1978. Obtaining confidence intervals for the risk ratio in cohort studies. Biometrics 34:469–474. https://doi.org/10.2307/2530610.

Khan, M. Z., S. Liu, Y. Ma, M. Ma, Q. Ullah, I. M. Khan, J. Wang, J. Xiao, T. Chen, A. Khan, and Z. Cao. 2023. Overview of the effect of rumen-protected limiting amino acids (methionine and lysine) and choline on the immunity, antioxidative, and inflammatory status of periparturient ruminants. Front. Immunol. 13:1042895. https://doi.org/10.3389/fimmu.2022.1042895.

Kies, A. K., L. H. De Jonge, P. A. Kemme, and A. W. Jongbloed. 2006. Interaction between protein, phytate, and microbial phytase. In vitro studies. J. Agric. Food Chem. 54:1753–1758. https://doi.org/10.1021/jf0518554.

Kim, J.-E., and H.-G. Lee. 2021. Amino acids supplementation for the milk and milk protein production of dairy cows. Animals (Basel) 11:2118. https://doi.org/10.3390/ani11072118.

Konishi, C., T. Matsui, W. Park, H. Yano, and F. Yano. 1999. Heat treatment of soybean meal and rapeseed meal suppresses rumen degradation of phytate phosphorus in sheep. Anim. Feed Sci. Technol. 80:115–122. https://doi.org/10.1016/S0377-8401(99)00044-9.

Krieg, J., E. Koenzen, N. Seifried, H. Steingass, H. Schenkel, and M. Rodehutscord. 2018. Prediction of CP and starch concentrations in ruminal in situ studies and ruminal degradation of cereal grains using NIRS. Animal 12:472–480. https://doi.org/10.1017/S1751731117001926.

Licitra, G., T. M. Hernandez, and P. J. Van Soest. 1996. Standardization of procedures for nitrogen fractionation of ruminant feeds. Anim. Feed Sci. Technol. 57:347–358. https://doi.org/10.1016/0377-8401(95)00837-3.

Liebe, D. M., J. L. Firkins, H. Tran, P. J. Kononoff, and R. R. White. 2018. Technical note: Methodological and feed factors affecting measurement of protein A, B, and C fractions, degradation rate, and intestinal digestibility of rumen-undegraded protein. J. Dairy Sci. 101:8046–8053. https://doi.org/10.3168/jds.2018-14837.

Madsen, J., and T. Hvelplund. 1994. Prediction of in situ protein degradability in the rumen. Results of a European ringtest. Livest. Prod. Sci. 39:201–212. https://doi.org/10.1016/0301-6226(94)90185-6.

Maxin, G., D. R. Ouellet, and H. Lapierre. 2013. Ruminal degradability of dry matter, crude protein, and amino acids in soybean meal, canola meal, corn, and wheat dried distillers grains. J. Dairy Sci. 96:5151–5160. https://doi.org/10.3168/jds.2012-6392.

McDonald, I. 1981. A revised model for the estimation of protein degradability in the rumen. J. Agric. Sci. 96:251–252. https://doi.org/10.1017/S0021859600032081.

Miranda, M. S., J. R. P. Arcaro, A. Saran Netto, S. L. Silva, M. G. Pinheiro, and P. R. Leme. 2019. Effects of partial replacement of soybean meal with other protein sources in diets of lactating cows. Animal 13:1403–1411. https://doi.org/10.1017/S1751731118002926.

Mjoun, K., K. F. Kalscheur, A. R. Hippen, and D. J. Schingoethe. 2010. Ruminal degradability and intestinal digestibility of protein and amino acids in soybean and corn distillers grains products. J. Dairy Sci. 93:4144–4154. https://doi.org/10.3168/jds.2009-2883.

NASEM. 2021. Nutrient Requirements of Dairy Cattle. 8th rev. ed. Animal Nutrition Series. The National Academies Press, Washington, DC.

O'Mara, F. P., J. J. Murphy, and M. Rath. 1997. The amino acid composition of protein feedstuffs before and after ruminal incubation and after subsequent passage through the intestines of dairy cows. J. Anim. Sci. 75:1941–1949. https://doi.org/10.2527/1997.7571941x.

Ørskov, E. R., and I. McDonald. 1979. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. J. Agric. Sci. 92:499–503. https://doi.org/10.1017/S0021859600063048.

Park, W.-Y., T. Matsui, C. Konishi, S.-W. Kim, F. Yano, and H. Yano. 1999. Formaldehyde treatment suppresses ruminal degradation of phytate in soyabean meal and rapeseed meal. Br. J. Nutr. 81:467–471. https://doi.org/10.1017/S0007114599000823.

Pesic, M. B., B. V. Vucelic-Radovic, M. B. Barac, and S. P. Stanojevic. 2005. The influence of genotypic variation in protein composition on emulsifying properties of soy proteins. J. Am. Oil Chem. Soc. 82:667–672. https://doi.org/10.1007/s11746-005-1126-x.

Piepho, H. P. 2012. A SAS macro for generating letter displays of pairwise mean comparisons. Commun. Biom. Crop Sci. 7:4–13.

Prattley, C. A., and D. W. Stanley. 1982. Protein-phytate interactions in soybeans. I. Localization of phytate in protein bodies and globoids. J. Food Biochem. 6:243–254. https://doi.org/10.1111/j.1745-4514.1982.tb00305.x.

Ray, P. P., J. Jarrett, and K. F. Knowlton. 2013. Effect of dietary phytate on phosphorus digestibility in dairy cows. J. Dairy Sci. 96:1156–1163. https://doi.org/10.3168/jds.2012-5851.

Ray, P. P., C. Shang, R. E. Pearson, and K. F. Knowlton. 2012. Disappearance of infused phytate from the large intestine of dairy heifers. J. Dairy Sci. 95:5927–5935. https://doi.org/10.3168/jds.2012-5363.

Rodehutscord, M., M. Kapocius, R. Timmler, and A. Dieckmann. 2004. Linear regression approach to study amino acid digestibility in broiler chickens. Br. Poult. Sci. 45:85–92. https://doi.org/10.1080/00071660410001668905.

Schwab, C. G., and G. A. Broderick. 2017. A 100-year review: Protein and amino acid nutrition in dairy cows. J. Dairy Sci. 100:10094–10112. https://doi.org/10.3168/jds.2017-13320.

Seifried, N., H. Steingass, N. Hoffmann, and M. Rodehutscord. 2017. In situ starch and crude protein degradation in the rumen and in vitro gas production kinetics of wheat genotypes. J. Anim. Physiol. Anim. Nutr. (Berl.) 101:779–790. https://doi.org/10.1111/jpn.12529.

Siegert, W., S. Kuenz, W. Windisch, and M. Rodehutscord. 2023. Amino acid digestibility and metabolizable energy of soybean meal of different origins in cecectomized laying hens. Poult. Sci. 102:102580. https://doi.org/10.1016/j.psj.2023.102580.

Sniffen, C. J., J. D. O'Connor, P. J. Van Soest, D. G. Fox, and J. B. Russell. 1992. A net carbohydrate and protein system for evaluating cattle diets: II. Carbohydrate and protein availability. J. Anim. Sci. 70:3562–3577. https://doi.org/10.2527/1992.70113562x.

Sommerfeld, V., S. Künzel, M. Schollenberger, I. Kühn, and M. Rodehutscord. 2018. Influence of phytase or myo-inositol supplements on performance and phytate degradation products in the crop, ileum, and blood of broiler chickens. Poult. Sci. 97:920–929. https://doi.org/10.3382/ps/pex390.

Steingass, H., G. Kneer, G. Wischer, and M. Rodehutscord. 2013. Variation of in situ rumen degradation of crude protein and amino acids and in vitro digestibility of undegraded feed protein in

rapeseed meals. Animal 7:1119–1127. https://doi.org/10.1017/S175173111300030X.

Susmel, P., B. Stefanon, C. R. Mills, and M. Candido. 1989. Change in amino acid composition of different protein sources after rumen incubation. Anim. Sci. 49:375–383. https://doi.org/10.1017/S0003356100032591.

Titze, N., Y.-P. Chi, E. Haese, J. Hartung, and M. Rodehutscord. 2023. Linkage of in situ ruminal crude protein degradation with ruminal degradation of amino acids and phytate from different soybean meals in dairy cows. https://doi.org/10.5281/zenodo.8329426.

Titze, N., J. Krieg, H. Steingass, and M. Rodehutscord. 2019. Variation of lupin protein degradation in ruminants studied in situ and using chemical protein fractions. Animal 13:709–717. https://doi.org/10.1017/S1751731118002124.

Titze, N., J. Krieg, H. Steingass, and M. Rodehutscord. 2021. In situ crude protein and starch degradation and in vitro evaluation of pea grains for ruminants. Arch. Anim. Nutr. 75:422–434. https://doi.org/10.1080/1745039X.2021.1994831.

VDLUFA. 2007. Handbuch der landwirtschaftlichen Versuchs- und Untersuchungsmethodik (VDLUFA-Methodenbuch), Vol. III Die chemische Untersuchung von Futtermitteln. VDLUFA Verlag, Darmstadt, Germany.

Wang, R., J. Liu, and S. Guo. 2018. Binding of phytate to soybean protein during the heat treatment of soymilk and its effect on protein aggregation. Food Hydrocoll. 84:368 378. https://doi.org/10.1016/j.foodhyd.2018.06.031.

Westreicher-Kristen, E., H. Steingass, and M. Rodehutscord. 2013. In situ ruminal degradation of amino acids and in vitro protein digestibility of undegraded CP of dried distillers' grains with solubles from European ethanol plants. Animal 7:1901–1909. https://doi.org/10.1017/S1751731113001730.

White, R. R., P. J. Kononoff, and J. L. Firkins. 2017. Technical note: Methodological and feed factors affecting prediction of ruminal degradability and intestinal digestibility of essential amino acids. J. Dairy Sci. 100:1946 1950. https://doi.org/10.3168/jds.2016-12008.

Wulf, M., and K.-H. Südekum. 2005. Effects of chemically treated soybeans and expeller rapeseed meal on in vivo and in situ crude fat and crude protein disappearance from the rumen. Anim. Feed Sci. Technol. 118:215–227. https://doi.org/10.1016/j.anifeedsci.2004.11.001.

Yiu, S. H., I. Altosaar, and R. G. Fulcher. 1983. The effects of commercial processing on the structure and microchemical organization of rapeseed. Food Struct. 2:165 173.

Zeller, E., M. Schollenberger, I. Kühn, and M. Rodehutscord. 2015. Hydrolysis of phytate and formation of inositol phosphate isomers without or with supplemented phytases in different segments of the digestive tract of broilers. J. Nutr. Sci. 4:e1. https://doi.org/10.1017/jns.2014.62.

Zilić, S. M., M. B. Barać, M. B. Pesić, S. D. Mladenović Drinić, D. D. Ignjatović-Micić, and M. B. Srebrić. 2011. Characterization of proteins from kernel of different soybean varieties. J. Sci. Food Agric. 91:60–67. https://doi.org/10.1002/jsfa.4148.

ORCIDS

N. Titze https://orcid.org/0000-0001-5992-6404
Y.-P. Chi https://orcid.org/0000-0003-2117-6606
E. Haese https://orcid.org/0000-0001-7795-1750
M. Rodehutscord https://orcid.org/0000-0003-3156-7889

4.3 *IN VITRO* STUDY

In vitro ruminal phytate degradation of rapeseed meal and soybean meal as affected by the provided amount of phytate

Abstract

The objective of the present study was to determine the phytate (**$InsP_6$**) degradation of rapeseed meal (**RSM**) and soybean meal (**SBM**) in response to different amounts of feed using an *in vitro* assay and to link the results to previous *in situ* and *in vivo* data obtained with the same batches of RSM and SBM. The RSM and SBM were incubated in amounts equivalent to 60, 120, and 180 mg of $InsP_6$ using a modified rumen simulation technique (**RUSITEC**) for 3, 6, 12, 24, and 48 h. The quantity of $InsP_6$ in the liquid samples (rumen fluid/buffer mixture and fluid in the fermenter vessel or beaker at the targeted incubation time) and the amounts of inositol phosphates (**$InsP_{3-6}$**) in the bag residues were determined. No $InsP_6$ was detected in any of the liquid samples. For bag residues, the amounts of $InsP_6$ and $InsP_5$ recovered at the targeted incubation time differed between RSM and SBM and among the incubated amounts of $InsP_6$. Trace amounts of $InsP_4$ were detected in the bag residues of the RSM, whereas $InsP_3$ was not detected in any bag residue. Significant interaction effects were observed for the degradation parameters and calculated effective degradation of $InsP_6$ (**$InsP_6$ED**). Based on regression analysis, the amount of $InsP_6$ED (**$InsP_6$ED(mg)**) increased by 0.83, 0.67, and 0.58 mg per each incremental mg of $InsP_6$ contained in RSM and by 0.94, 0.88, and 0.82 mg per each incremental mg of $InsP_6$ contained in SBM at rumen passage rates (k) of 0.02, 0.05, and 0.08 h^{-1}, respectively. However, the percentage of effective degradation of $InsP_6$ (**$InsP_6$ED(%)**) increased with the incubation amount of $InsP_6$ from 120 to 180 mg for the RSM, whereas the $InsP_6$ED(%) of the SBM increased for amounts of up to 120 mg $InsP_6$ and then stabilised for the amount of 180 mg $InsP_6$ at k = 0.05 and 0.08 h^{-1}. These results suggest that it is more suitable to evaluate $InsP_6$ degradation based on the amount of $InsP_6$ supplied rather than only relative values. The calculated $InsP_6$ED(%) in the present study showed high similarity to the preliminary *in situ* and *in vivo* data, indicating that the principal findings of this *in vitro* study can be applied to rumen degradation.

Introduction

Plant seeds and their processing by-products are common feedstuffs in ruminant nutrition and contain large amounts of phosphorus (**P**), which is predominantly bound in the form of phytate, the salt form of *myo*-inositol 1,2,3,4,5,6 hexakis dihydrogen phosphate (**$InsP_6$**). The cleavage of P from $InsP_6$, which is catalysed by phytases, enables P absorption in the intestine and further metabolic utilisation by animals. Phytases produced by rumen microorganisms are highly effective in $InsP_6$ degradation, thus providing ruminants with a high potential to utilise $InsP_6$-P (Raun et al. 1956; Yanke et al. 1998; Haese et al. 2017a). However, the extent of ruminal $InsP_6$ degradation may not be complete, and a wide variation of ruminal $InsP_6$ degradation has been found among feedstuffs (48–90% at a rumen passage rate of 0.08 h^{-1}) (Haese et al., 2020). Incomplete ruminal $InsP_6$ degradation may cause increased excretion of $InsP_6$ because post-ruminal $InsP_6$ degradation is negligible compared to ruminal degradation (Chi et al., 2022). Degradation of $InsP_6$ in the large intestine specifically is not affected by the amount of $InsP_6$ entering the organ (Ray et al., 2012, 2013).

Evaluation of the degradation of $InsP_6$ from different feedstuffs has been performed predominantly by including a certain amount of test feedstuffs for incubation or diet formulation, disregarding the different $InsP_6$ concentrations of the feedstuffs (Morse et al., 1992; Haese et al., 2017b, 2020; Chi et al., 2022). This may be confounded by the amount of supplied $InsP_6$ as the concentration in different test feedstuffs varied, and the amount of dietary $InsP_6$ has been suggested to influence the extent of ruminal $InsP_6$ degradation (Ray et al., 2013). The $InsP_6$ degradation is commonly expressed as a relative value (percentage or g/kg of dry matter (**DM**)) (Park et al., 1999; Martín-Tereso et al., 2009; Brask-Pedersen et al., 2011; Ray et al., 2013; Haese et al., 2020). This may not reflect the quantity of $InsP_6$ degraded in the rumen. In a previous study (Chi et al., 2022), percentage ruminal $InsP_6$ disappearance was higher in wethers fed a diet containing soybean meal (**SBM**) than in those fed a diet containing

the same amount of rapeseed meal (**RSM**). In contrast, the amount of $InsP_6$ that disappeared in the rumen was lower with SBM feeding.

In the present study, we aimed to achieve a better understanding of how different amounts of $InsP_6$ in the feed can influence $InsP_6$ degradation in different feedstuffs and whether an upper limit of $InsP_6$ degradation exists. We selected RSM and SBM from the same batches as used in a previous study (Chi et al., 2022) to enable data linkage. We adopted an *in vitro* approach, as it enables the investigation of treatments and factor combinations on a larger scale than *in vivo* and *in situ* methods. *In vitro* incubation has previously been used to study effects on $InsP_6$ degradation (Morse et al., 1992; Brask-Pedersen et al., 2011; Haese et al., 2014, 2017a). We hypothesised that the *in vitro* $InsP_6$ degradation in response to the amount of $InsP_6$ would differ between RSM and SBM. We also analysed less-phosphorylated inositol phosphates (**$InsP_{3\text{-}5}$**) to assess whether the pattern of $InsP_{3\text{-}5}$ during incubation changed with the amount of $InsP_6$ supplied by RSM and SBM.

Material and methods

Animals and their diets

Four ruminally fistulated lactating Jersey cows were used as ruminal fluid donors for the *in vitro* incubation. The animals were housed, fed, and treated according to German animal welfare regulations, and their use in this study was approved by the Regierungspräsidium Stuttgart, Germany (approval code: V352/18 TE). The feed was provided as a total mixed ration with a 32% concentrate mixture (consisting of 27% winter barley, 29% maize, 25% faba beans, and 19% peas), 7.3% RSM, 0.6% soybean cake, 18% maize silage, 22% grass silage, 15% hay, and 2.5% straw on a DM basis. This ration contained 6.6 MJ/kg DM of net energy for lactation. The concentrations of P and crude protein (**CP**) were 4.4 g/kg DM and 142 g/kg DM, respectively (Table 4.3.1). The average DM intake of the cows was 19.3 kg/d.

Feed samples

The RSM and SBM were ground to pass through a 1-mm sieve and incubated in amounts equivalent to an $InsP_6$ amount of 60, 120, and 180 mg (**RSM-60**, **RSM-120**, **RSM-180** and **SBM-60**, **SBM-120**, **SBM-180**). To achieve the respective $InsP_6$ amount, 3, 6, and 9 g RSM and 4.5, 9, and 14 g SBM were weighed into polyester bags (10 × 20 cm, pore size of 50 µm, ANKOM Technology, USA). To maintain an adequate ratio of sample size to bag surface area, as suggested by Diao et al. (2020), incubation amounts greater than 8 g were equally divided into two polyester bags. The bags were stored at 4 °C prior to incubation.

For chemical analysis, feed samples were ground to pass through a 0.5-mm sieve (Ultra Centrifugal Mill ZM 200, Retsch GmbH, Haan, Germany), and a portion of each was further pulverised using a mixer mill (Type MM400, Retsch GmbH, Haan, Germany). The analytical values of the RSM and SBM are shown in Table 4.3.1. The analysed concentration of $InsP_6$ was 22.0 and 12.6 g/kg DM for RSM and SBM, respectively. The $InsP_5$ concentration was 3.8 g/kg DM for RSM and 2.0 g/kg DM for SBM. Only traces of $InsP_4$ and no $InsP_3$ were detected in either oilseed meal. The concentrations of CP, neutral detergent fibre without residual ash (**NDFom**), and acid detergent fibre without residual ash (**ADFom**) were 360 g/kg DM vs 506 g/kg DM, 316 g/kg vs 165 g/kg DM, and 245 g/kg vs 73 g/kg DM for RSM and SBM, respectively.

In vitro procedure

A modified rumen simulation technique (**RUSITEC**), described in detail and illustrated by Künzel et al. (2022), was used. The incubation times were 3, 6, 12, 24, and 48 h. For each $InsP_6$ amount and incubation time, RSM and SBM were incubated in three replicate fermenter vessels, which were randomly allocated to eight incubation runs, with 12 fermenter vessels in the first seven runs and six fermenter vessels in the last run. At the beginning of each incubation

run, rumen fluid was collected in the morning just before fresh feed was offered to the cows. Rumen fluid was strained through four layers of cheesecloth and pooled using equal amounts from the donor animals. The pooled rumen fluid was flushed with CO_2 and stirred at 39 °C until the start of the incubation (Künzel et al., 2022). A P-free buffer solution was prepared according to Komisarczuk et al. (1987) to mimic saliva and mixed with rumen fluid at a ratio of 1:1. Each fermenter vessel was filled with 800 mL rumen fluid/buffer mixture, and a feed container including bags with either RSM or SBM was inserted for incubation. The fermenter vessels were closed and connected to a lift motor, which generated continuous vertical movement (10–12 strokes/min) to simulate rumen motility. The system was maintained at a temperature between 38.5 and 39.0 °C without continuous buffer infusion or gas collection. At the end of each targeted incubation time, the pH in the fermenter fluid was measured, and the bags were removed, rinsed with 200 mL cold double-distilled water, and immediately frozen at −20 °C. The frozen bags were lyophilised and weighed, and the residues were collected and pulverised (Type MM400; Retsch GmbH, Haan, Germany).

The initial 0-h values were obtained by incubating the respective amounts of RSM and SBM in three 1-L beakers filled with 800 mL rumen fluid/buffer mixture under stirring and CO_2 flushing for 1 min (Haese et al. 2017a). The beakers were randomised into eight incubation runs. The bags were processed as described previously for the other bags.

Liquid samples, including rumen fluid/buffer mixture and fluid in the fermenter vessel or beaker at the targeted incubation time, were collected in 10 mL plastic tubes (Greiner Bio-One GmbH, Frickenhausen, Germany) and immediately frozen at −20 °C until further processing; five replicates were used for each.

Chemical analysis

Dry matter and crude nutrients were analysed using methods approved for use in Germany (Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten

(VDLUFA), 2012). The RSM, SBM, bag residues, and cow rations were analysed for DM (Method 3.1). Additionally, RSM, SBM, and rations were analysed for crude ash (method 8.1), CP (method 4.1.1), NDFom (method 6.5.1), and ADFom (method 6.5.2). Total P was determined by modified sulfuric and nitric acid wet digestion according to Boguhn et al. (2009), followed by measurement using an inductively coupled plasma optical emission spectrometer (Zeller et al., 2015). The concentrations of $InsP_{3-6}$ in the RSM, SBM, ration, and bag residues were determined by extracting 0.05 g of each sample using the method of Zeller et al. (2015), with modifications described by Sommerfeld et al. (2018). In brief, samples were extracted with a solution of 0.2 M EDTA and 0.1 M sodium fluoride at 4 °C (pH 8) for 30 min. After two extractions, followed by centrifugation at 12,000 × g for 15 min, 1 mL sample was collected, centrifuged at 14,000 × g for 15 min, and filtered prior to $InsP_{3-6}$ detection using high-performance ion chromatography (ICS-3000 system, Dionex, Idstein, Germany). In this assay, the enantiomers could not be distinguished.

The frozen samples were thawed shortly before $InsP_6$ extraction for the analysis of $InsP_6$ in the liquid samples, using the modified method of Haese et al. (2017a). Briefly, 0.3725 g EDTA was weighed into a centrifuge tube, 0.25 mL of cold 0.5 M sodium hydroxide-sodium-fluoride solution was added under stirring, and 4.75 mL of liquid samples were added thereafter. Samples were extracted once for 15 min, and 2 mL of the sample was collected for centrifugation at 14,000 × g for 15 min. The supernatant was filtered through a syringe filter into a tube and centrifuged again for 30 min before being measured as described above.

Calculations and statistics

The quantity of $InsP_6$ in the liquid samples was calculated by multiplying the concentration of $InsP_6$ with the quantity of liquid in the fermenter vessels or beakers at any incubation time (t). The total amount of $InsP_{3-6}$ in the bag residues at time t was calculated by multiplying the analysed concentrations of $InsP_{3-6}$ by the amount of DM in the bags. The degraded amount of

$InsP_{3-6}$ from the bag residues at time t was calculated as the difference between the measured amounts before and after incubation.

Two exponential models with or without lag time (**Lag**) (Ørskov and McDonald, 1979; McDonald, 1981) were used to fit the data of cumulative $InsP_6$ degradation over incubation time and to obtain degradation parameters ***a*** (mg; rapidly degradable amount), ***b*** (mg; potentially degradable amount), ***c*** (h^{-1}; degradation rate constant of *b*), and Lag (h, time until the initiation of degradation of *b*) of $InsP_6$ using GraphPad Prism software (version 5.0, GraphPad Software Inc., CA, USA). Maximum degradation was constrained to 60, 120, and 180 mg. Based on the Akaike information criterion, the model without lag resulted in a better fit.

$$InsP6\ degradation\ [mg] = a + b \times (1 - e^{-ct})$$

The effective degradation of $InsP_6$ (**$InsP_6$ED**) was calculated using the equation suggested by McDonald (1981), assuming ruminal passage rates (*k*) of 0.02, 0.05, and 0.08 h^{-1}.

$$InsP6ED\ = a + [(b \times c)/(c + k)]$$

The parameters *a*, *b*, and *c* and the $InsP_6$ED values were calculated for each oilseed meal and $InsP_6$ amount using three replicates. Data were statistically analysed using a two-factorial approach with the MIXED procedure in SAS (version 9.4; SAS Institute Inc., Cary, NC, USA) using the following model:

$Y_{ij} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + e_{ij}$

where Y_{ij} is the trait, μ the overall mean, α_i the fixed effect of oilseed meal, β_j the fixed effect of $InsP_6$ amount, $(\alpha\beta)_{ij}$ the interaction between the fixed effects, and eij as residual error. Run and fermenter vessels or beakers were considered random effects. Linear regression was performed using the REG procedure to evaluate the slope of the relationship between the incubated amounts of $InsP_6$ and $InsP_6$ED. Statistical significance was set at $P < 0.05$.

Results

Inositol phosphates

$InsP_6$ was below the detection limit (< 0.05 µmol/mL) in all liquid samples (rumen fluid/buffer mixture and fluid in the fermenter vessel or beaker at the target incubation time). The amount of $InsP_6$ recovered from the bag residue at the targeted incubation time differed between RSM and SBM and among the incubated amounts (data are shown without statistical analysis in Figure 4.3.1). $InsP_6$ from the bag residues of the RSM slightly increased, independent of the incubated amount, between 0 and 3 h, and decreased to approximately 60 mg after 12 h of incubation for RSM-120 and RSM-180. After 24 h of incubation, $InsP_6$ was detected at a similar level (10 ± 4 mg) at all incubated amounts, and $InsP_6$ was reduced to less than 1 mg at all incubated $InsP_6$ amounts after 48 h of incubation of RSM. When SBM was incubated, $InsP_6$ was considerably reduced from 42, 83, and 129 mg to 23, 27, and 34 mg in SBM-60, SBM-120, and SBM-180, respectively, between 0 and 6 h. After 24 h of incubation, less than 1 mg of $InsP_6$ from the SBM was detected in the bag residues for all the incubated $InsP_6$ amounts.

The amount of $InsP_5$ in the bag residue followed a similar pattern to that of $InsP_6$ (Figure 4.3.1). After 12 h of incubation, less than 0.5 mg $InsP_5$ was detected for SBM-120 and SBM-180, whereas a remarkable amount of $InsP_5$ was still present in the bag residues of the RSM at this point in time (5.2, 11.2, and 11.9 mg for RSM-60, RSM-120, and RSM-180, respectively). No $InsP_5$ was found after 48 h of incubation in RSM with any of the incubated $InsP_6$ amounts. A trace amount (≤ 1 mg) of $InsP_4$ was detected in the bag residues of the RSM until 12 h of incubation, but it was not detectable in the majority of bag residues of the SBM. $InsP_3$ was not detected in any bag residue.

Degradation parameters and calculated effective degradation of $InsP_6$

Significant interaction effects were observed for the degradation parameters and calculated effective degradation of $InsP_6$ (Table 4.3.2). Parameter *a* did not significantly differ between RSM-60 and SBM-60 (4.6 vs 14.9 mg) but increased to a greater extent for SBM than for RSM with the increasing amount of $InsP_6$. The highest value of parameter *b* was 155 mg for RSM-180, and the lowest value was 45 mg for SBM-60, which did not differ significantly from 55 mg for RSM-60. Compared with the RSM, the parameter *c* of the SBM was significantly higher and increased from 0.122 h^{-1} for SBM-60 to 0.168 h^{-1} for SBM-120 (RSM-60: 0.054 h^{-1}; RSM-120: 0.053 h^{-1}; RSM-180: 0.068 h^{-1}, $P > 0.05$). The effective degradation of $InsP_6$, expressed as the amount (**$InsP_6$ED(mg)**), increased with the incubated amount of $InsP_6$, which was more pronounced for SBM than for RSM. Based on the regression analysis, $InsP_6$ED(mg) increased by 0.83, 0.67, and 0.58 mg per each incremental mg of $InsP_6$ contained in RSM ($R^2 \geq 0.98$; RMSE ≤ 4.15) and by 0.94, 0.88, and 0.82 mg per each incremental mg of $InsP_6$ contained in SBM ($R^2 > 0.99$; RMSE ≤ 1.96) at k = 0.02, 0.05, and 0.08 h^{-1}, respectively (Table 4.3.3).

The percentage of effective degradation of $InsP_6$ (**$InsP_6$ED(%)**) increased with the incubated amount of $InsP_6$ from 120 to 180 mg for RSM, whereas the $InsP_6$ED(%) of SBM increased up to 120 mg for $InsP_6$ and did not change further up to 180 mg for $InsP_6$ at k = 0.05 and 0.08 h^{-1}. Regardless of the incubated $InsP_6$ amount, $InsP_6$ED(%) was higher in SBM than in RSM (Table 4.3.2).

Discussion

$InsP_6$ degradation in the liquid phase of the fermenter

Consistent with the results of previous studies, $InsP_6$ was not detected in any of the liquid samples (< 33 mg/L). In an *in vivo* experiment, Chi et al. (2022) analysed lyophilised rumen

samples obtained from wethers and detected $InsP_6$ only in the particulate matter but not in the liquid phase (< 0.13 g/kg DM in the supernatant of the filtered rumen fluid). In the *in vitro* experiment by Haese et al. (2017a), very low levels of $InsP_6$ were calculated in the rumen fluid/buffer mixture prepared for the incubation (6.8 mg/L for 'high P' diet) and fermenter fluids after 3 h of incubation (< 5.3 mg/L). These data indicated that $InsP_6$, which may have entered the liquid phase of the fermenter, was very rapidly degraded.

$InsP_6$ degradation in bag residues

Significant interactions between degradation parameters and $InsP_6ED$ confirmed our hypothesis that $InsP_6$ degradation in response to the amount of incubated $InsP_6$ differed between the two oilseed meals. A comparison of the effect of the amount of $InsP_6$ on $InsP_6$ degradation from different feeds with that reported in the literature was not possible. However, the average value of $InsP_6ED_5$(%) over the three incubated amounts of $InsP_6$ in the present study was 83% for SBM (79, 84, and 85% for SBM-60, SBM-120, and SBM-180, respectively) and 59% for RSM (56, 56, and 64% for RSM-60, RSM-120, and RSM-180, respectively). This difference is consistent with an *in situ* study by Haese et al. (2020), where $InsP_6ED_5$(%) was 76% for SBM and 59% for RSM. The *in vitro* calculated $InsP_6ED_5$(%) was similar to the value determined in an *in situ* study using the same batches of oilseed meals (Chi et al., 2022), which was 85% for SBM and 64% for RSM. These similarities suggest that the effective degradation estimated using the present *in vitro* approach is comparable to that estimated using the *in situ* method, indicating the potential for establishing a standard *in vitro* procedure.

For both oilseed meals, $InsP_6$ degradation was almost complete after 48 h of incubation, independent of the incubated amount of $InsP_6$ and $InsP_6$ was the most abundant inositol phosphate, without the accumulation of $InsP_5$, $InsP_4$, or $InsP_3$. The ability of the mixed microbial population for $InsP_6$ degradation from feed was remarkably high. In accordance with

the results of Ray et al. (2013), in which greater ruminal $InsP_6$ degradation was found in dairy cows fed a diet with a higher $InsP_6$-P content (53, 30, and 16 g/d for high, medium, and low $InsP_6$-P diets, respectively), the calculated $InsP_6$ED(mg) increased linearly with increasing $InsP_6$ amounts for both RSM and SBM (Table 4.3.3). However, the increase in $InsP_6$ED(mg) upon increasing the amount of $InsP_6$ was more pronounced in SBM than in RSM. Compared to SBM, the parameter *a* of $InsP_6$ increased to a lesser extent with increasing incubated $InsP_6$ amounts, and the degradation of $InsP_6$ during incubation was overall slower for RSM. Moreover, the increase in $InsP_6$ED(mg) estimated by linear regression was reduced to a greater extent for RSM (0.25 mg) than for SBM (0.12 mg) when the presumed rumen passage rate *k* increased from 0.02 to 0.08 h^{-1}. Hence, the differences between RSM and SBM influencing solubility and degradation kinetics were likely the reason for the incongruent effects of the incubated $InsP_6$ amount observed between the oilseed meals.

Rapeseed and soybeans differ in the internal structure of their protein storage vacuoles (**PSV**), and $InsP_6$ is located in most mature seeds. Although $InsP_6$ is distributed mostly homogeneously in soybeans (Prattley and Stanley, 1982), it is confined to additional globoids in rapeseed (Yiu et al., 1983). Within the PSV, $InsP_6$ is present predominantly as Ca- and Mg-phytate in rapeseed (Gillberg and Törnell, 1976) and has a generally lower solubility than the main storage form of K-phytate in soybeans (Prattley and Stanley, 1982; Lott et al., 1985). The presence of polyvalent cations also favours the formation of larger protein-phytate complexes (Hídvégi and Lásztity, 2002). The additional structure and binding of $InsP_6$ with polyvalent cations in rapeseed may have lowered the accessibility of microbial phytases to their substrates for RSM, slowing down the rate of $InsP_6$ degradation and limiting $InsP_6$ degradation over time.

In addition to the localisation and binding form of $InsP_6$ in the seeds, incubating RSM and SBM may have contributed to the differences in the rate and amount of nutrients and energy supply for the microbiota in the *in vitro* system. Yanke et al. (1998) inoculated rumen fluid

obtained from two rumen-fistulated steers with fermentable carbohydrate sources and isolated bacterial strains of *Prevotella ruminicola*, *Megasphaera elsdenii*, *Selenomonas ruminantium*, *Mitsuokella multiacidus* and *Treponema* spp. that exhibited phytase activity. Lan et al. (2002b) further isolated the phytase-producing bacterial species *Mitsuokella jalaludinii* from *in vitro* rumen fluid inoculation. However, Lan et al. (2002a) observed differences in phytase production in *Mitsuokella jalaludinii* in different media compositions, which may be attributed to the concentration of the substrate phytate and the release of carbohydrates and amino acids. In comparison with SBM, RSM contains a higher fraction of fibres and proteins that are more resistant to microbial fermentation, as indicated by the lower effective degradation of CP (Chi et al., 2022) and the slower and less complete degradation of DM (data not shown). This may have resulted in a slower release and less efficient supply of carbon and nitrogen sources that are essential for microbial growth and enzyme production (Lan et al., 2002a) and, consequently, slower degradation of $InsP_6$ from RSM. Nevertheless, the $InsP_6$ amounts in this study were achieved solely by incubating different amounts of oilseed meal without ensuring the same amount of other nutrients. The total DM amount of the incubated feed was higher and increased with increasing $InsP_6$ incubation amounts, to a greater extent for SBM than for RSM. Hence, the proliferation and function of phytase-producing microorganisms may also have been favoured by a greater total increase in sources such as nitrogen from SBM. Increasing the amount of incubated oilseed meal in the *in vitro* system also resulted in a slightly greater reduction of the pH in the fermenter for SBM compared to RSM, although the range before and after incubation was between pH 6 and 7 (data not shown); thus, the influence of slight pH change on microbial phytase activity could not be ruled out (Haros et al., 2005; Lamid et al., 2018).

Because of the counteracting degradation kinetics for RSM between 0 and 3 h of incubation in this study, we excluded the 3 h values for the computation of the degradation

parameters and calculation of $InsP_6$ED. However, the values of $InsP_6$ED and their significance levels were similar to those obtained by including the 3 h values. The higher amount of $InsP_6$ at 3 h compared to that at 0 h of incubation in RSM for all incubated $InsP_6$ amounts is similar to the results reported by Haese et al. (2017a) and Haese et al. (2017b) using *in vitro* and *in situ* assays, respectively. Previously, $InsP_6$ was found to be associated with denatured protein aggregates formed during RSM production (Yiu et al., 1983). Thus, the increased $InsP_6$ concentration was ascribed to the prerequisite degradation of associated components prior to microbial $InsP_6$ degradation (Haese et al., 2017b). In addition, insoluble protein-phytate complexes have been reported to lower the extractability of $InsP_6$ (Serraino and Thompson, 1984; Tzeng et al., 1990). More $InsP_6$ may have been extracted and determined after 3 h of incubation because of the increased accessibility of $InsP_6$ through the intrinsic structural changes in the rapeseed protein polymer during incubation (Xin and Yu, 2013).

Comparison of $InsP_6$ degradation between feeds and diets should be conducted with caution. In a study by Chi et al. (2022), the ruminally degraded amount of $InsP_6$ was higher in wethers fed a RSM-containing diet with 5.9 g/d of $InsP_6$ than in those fed a SBM-containing diet with 3.9 g/d of $InsP_6$. In the present study, SBM generally demonstrated a higher $InsP_6$ED(mg) than RSM at the same incubated amount of $InsP_6$, whereas it was not the case when RSM was incubated with a higher amount of $InsP_6$. This demonstrates the importance of comparing feeds based on the same amount of $InsP_6$. In addition, similar to the pattern of parameter *c*, effective rumen degradation was not increased by incubating 180 mg of $InsP_6$ for SBM and did not differ between 60 and 120 mg of $InsP_6$ incubation for RSM when expressed as $InsP_6$ED(%). However, a more rapid decline and a greater degraded amount of $InsP_6$ was observed with increasing $InsP_6$ incubation amount over the course of incubation. This implies that the relative values may not reflect the real dynamics of quantitative $InsP_6$ degradation, as

the incubated and maximal degradation amounts of $InsP_6$ differed among the treatments in this study.

In conclusion, our results show that the effects of $InsP_6$ amount on *in vitro* $InsP_6$ degradation differed between oilseed meals, probably because of the internal structure and nutrient composition of the feed. The calculated $InsP_6$ED(mg) increased linearly with increasing $InsP_6$ amount in the feed, but $InsP_6$ED(%) did not. This suggested comparing $InsP_6$ degradation based on the amount of $InsP_6$ in the feed and expressed as the amount of $InsP_6$ instead of a relative value. The high similarity of findings from the present study with preliminary *in situ* and *in vivo* data indicates that the principal findings of this work also apply to rumen degradation.

References

Boguhn, J., Baumgärtel, T., Dieckmann, A., Rodehutscord, M., 2009. Determination of titanium dioxide supplements in different matrices using two methods involving photometer and inductively coupled plasma optical emission spectrometer measurements. Archives of Animal Nutrition 63, 337–342.

Brask-Pedersen, D.N., Glitsø, L.V., Skov, L.K., Lund, P., Sehested, J., 2011. Effect of exogenous phytase on feed inositol phosphate hydrolysis in an in vitro rumen fluid buffer system. Journal of Dairy Science 94, 951–959.

Chi, Y-P., Haese, E., Rodehutscord, M., 2022. Ruminal and post-ruminal phytate degradation of diets containing rapeseed meal or soybean meal. Archives of Animal Nutrition 76, 233–247.

Diao, X., Dang, S., Liu, S., Jing, L., Wang, Y., Zhang, W., 2020. Determination of the appropriate ratio of sample size to nylon bag area for in situ nylon bag technique evaluation of rumen digestibility of feedstuffs in sheep. Livestock Science 241, 104254.

Gillberg, L., Törnell, B., 1976. Preparation of rapeseed protein isolates. Dissolution and precipitation behavior of rapeseed proteins. Journal of Food Science 41, 1063–1069.

Haese, E., Krieg, J., Grubješić, G., Feyder, A., Rodehutscord, M., 2020. Determination of in situ ruminal degradation of phytate phosphorus from single and compound feeds in dairy cows using chemical analysis and near-infrared spectroscopy. Animal 14, 1461–1471.

Haese, E., Lengowski, M., Gräter, E., Föll, A., Möhring, J., Steingass, H., Schollenberger, M., Rodehutscord, M., 2017a. Ruminal phytate degradation of maize grain and rapeseed meal in vitro and as affected by phytate content in donor animal diets and inorganic phosphorus in the buffer. Journal of Animal Physiology and Animal Nutrition 101, 868–880.

Haese, E., Möhring, J., Steingass, H., Schollenberger, M., Rodehutscord, M., 2017b. Effect of dietary mineral phosphorus and phytate on in situ ruminal phytate disappearance from different concentrates in dairy cows. Journal of Dairy Science 100, 3672–3684.

Haese, E., Müller, K., Steingass, H., Schollenberger, M., Rodehutscord, M., 2014. Effects of mineral and rapeseed phosphorus supplementation on phytate degradation in dairy cows. Archives of Animal Nutrition 68, 478–491.

Haros, M., Bielecka, M., Sanz, Y., 2005. Phytase activity as a novel metabolic feature in Bifidobacterium. FEMS Microbiology Letters 247, 231–239.

Hídvégi, M., Lásztity, R., 2002. Phytic acid content of cereals and legumes and interaction with proteins. Periodica Polytechnica Chemical Engineering 46, 59–64.

Komisarczuk, S., Merry, R.J., McAllan, A.B., 1987. Effect of different levels of phosphorus on rumen microbial fermentation and synthesis determined using a continuous culture technique. British Journal of Nutrition 57, 279–290.

Künzel, S., Yergaliyev, T., Wild, K.J., Philippi, H., Petursdottir, A.H., Gunnlaugsdottir, H., Reynolds, C.K., Humphries, D.J., Camarinha-Silva, A., Rodehutscord, M., 2022. Methane reduction potential of brown seaweeds and their influence on nutrient

degradation and microbiota composition in a rumen simulation technique. Frontiers in Microbiology 13, 889618.

Lamid, M., Al-Arif, A., Asmarani, O., Warsito, S.H., 2018. Characterization of phytase enzymes as feed additive for poultry and feed. IOP Conference Series: Earth and Environmental Science 137, 012009.

Lan, G.Q., Abdullah, N., Jalaludin, S. Ho, Y.W., 2002a. Optimization of carbon and nitrogen sources for phytase production by Mitsuokella jalaludinii, a new rumen bacterial species. Letters in Applied Microbiology 35, 157–161.

Lan, G.Q., Ho, Y.W., Abdullah, N., 2002b. Mitsuokella jalaludinii sp. nov., from the rumens of cattle in Malaysia. International Journal of Systematic and Evolutionary Microbiology 52, 713–718.

Lott, J.N.A., Randall, P.J., Goodchild, D.J., Craig, S., 1985. Occurrence of globoid crystals in cotyledonary protein bodies of Pisum sativum as influenced by experimentally induced changes in Mg, Ca and K contents of seeds. Functional Plant Biology 12, 341.

Martín-Tereso, J., Gonzalez, A., Van Laar, H., Burbano, C., Pedrosa, M.M., Mulder, K., den Hartog, L.A., Verstegen, M.W.A., 2009. In situ ruminal degradation of phytic acid in formaldehyde-treated rice bran. Animal Feed Science and Technology 152, 286–297.

McDonald, I., 1981. A revised model for the estimation of protein degradability in the rumen. Journal of Agricultural Science 96, 251–252.

Morse, D., Head, H.H., Wilcox, C.J., 1992. Disappearance of phosphorus in phytate from concentrates in vitro and from rations fed to lactating dairy cows. Journal of Dairy Science 75, 1979–1986.

Ørskov, E.R., McDonald, I., 1979. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. The Journal of Agricultural Science 92, 499–503.

Park, W-Y., Matsui, T., Konishi, C., Kim, S-W., Yano, F., Yano, H., 1999. Formaldehyde treatment suppresses ruminal degradation of phytate in soyabean meal and rapeseed meal. British Journal of Nutrition 81, 467–471.

Prattley, C.A., Stanley, D.W., 1982. Protein-phytate interactions in soybeans. I. Localization of phytate in protein bodies and globoids. Journal of Food Biochemistry 6, 243–254.

Raun, A., Cheng, E., Burroughs, W., 1956. Ruminant nutrition, phytate phosphorus hydrolysis and availability to rumen microorganisms. Journal of Agricultural and Food Chemistry 4, 869–871.

Ray, P.P., Jarrett, J., Knowlton, K.F., 2013. Effect of dietary phytate on phosphorus digestibility in dairy cows. Journal of Dairy Science 96, 1156–1163.

Ray, P.P., Shang, C., Pearson, R.E., Knowlton, K.F., 2012. Disappearance of infused phytate from the large intestine of dairy heifers. Journal of Dairy Science 95, 5927–5935.

Serraino, M.R., Thompson, L.U., 1984. Removal of phytic acid and protein-phytic acid interactions in rapeseed. Journal of Agricultural and Food Chemistry 32, 38–40.

Sommerfeld, V., Künzel, S., Schollenberger, M., Kühn, I., Rodehutscord, M., 2018. Influence of phytase or myo-inositol supplements on performance and phytate degradation products in the crop, ileum, and blood of broiler chickens. Poultry Science 97, 920–929.

Tzeng, Y-M., Diosady, L.L., Rubin, L.J., 1990. Production of canola protein materials by alkaline extraction, precipitation, and membrane processing. Journal of Food Science 55, 1147–1151.

Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten (VDLUFA), 2012. Handbuch der Landwirtschaftlichen Versuchs-und Untersuchungsmethodik (VDLUFA-Methodenbuch), Bd. III: Die chemische Untersuchung von Futtermitteln. VDLUFA-Verlag, Darmstadt, Germany.

Xin, H., Yu, P., 2013. Detect changes in protein structure of carinata meal during rumen fermentation in relation to basic chemical profile and comparison with canola meal using ATR–FT/IR molecular spectroscopy with chemometrics. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 112, 318–325.

Yanke, L.J., Bae, H.D., Selinger, L.B., Cheng, K.J., 1998. Phytase activity of anaerobic ruminal bacteria. Microbiology 144, 1565–1573.

Yiu, S.H., Altosaar, I., Fulcher, R.G., 1983. The effects of commercial processing on the structure and microchemical organization of rapeseed. Food Structure 2, 165–173.

Zeller, E., Schollenberger, M., Kühn, I., Rodehutscord, M., 2015. Hydrolysis of phytate and formation of inositol phosphate isomers without or with supplemented phytases in different segments of the digestive tract of broilers. Journal of Nutritional Science 4, e1.

Table 4.3.1

Chemical composition of experimental feed and donor animal diet (g/kg DM)

	RSM	SBM	Donor animal diet[1]
Crude protein	360	506	142
Crude ash	88	80	79
NDFom	316	165	377
ADFom	245	73	236
P	11.6	7.1	4.4
Inositol phosphates			
$InsP_6$	22.0	12.6	5.7
$InsP_5$	3.8	2.0	0.2
$InsP_4$	0.1	0.1	n.d.

Abbreviations: DM = dry matter; RSM = rapeseed meal; SBM = soybean meal; NDFom = neutral detergent fibre expressed exclusive of residual ash; ADFom = acid detergent fibre expressed exclusive of residual ash; n.d. = not detectable.

[1] Donor animal diet was provided as total mixed ration.

Table 4.3.2

Effect of phytate ($InsP_6$) amount on *in vitro* degradation parameters and effective degradation of $InsP_6$ in the bag residues of the oilseed meals[1].

	RSM			SBM			SEM	*P*-value		
	60	120	180	60	120	180		Meal	Amount	Meal × amount
a (mg)	4.6^{c}	11.1^{c}	24.6^{b}	14.9^{bc}	37.1^{a}	46.9^{a}	2.48	<0.0001	<0.0001	0.002
b (mg)	55^{e}	109^{c}	155^{a}	45^{e}	83^{d}	133^{b}	2.5	<0.0001	<0.0001	0.014
c (h^{-1})	0.054^{c}	0.053^{c}	0.068^{c}	0.122^{b}	0.168^{a}	0.189^{a}	0.0086	<0.0001	0.002	0.018
$InsP_6ED_2$ (mg)	45^{e}	90^{d}	145^{b}	54^{e}	111^{c}	167^{a}	0.6	<0.0001	<0.0001	<0.0001
$InsP_6ED_5$ (mg)	33^{f}	67^{d}	114^{b}	47^{e}	101^{c}	152^{a}	0.9	<0.0001	<0.0001	<0.0001
$InsP_6ED_8$ (mg)	27^{e}	54^{c}	96^{b}	42^{d}	93^{b}	140^{a}	1.0	<0.0001	<0.0001	<0.0001
$InsP_6ED_2$ (%)	75^{d}	75^{d}	81^{c}	90^{b}	93^{ab}	93^{a}	0.6	<0.0001	<0.0001	0.008
$InsP_6ED_5$ (%)	56^{d}	56^{d}	64^{c}	79^{b}	84^{a}	85^{a}	0.9	<0.0001	<0.0001	0.007
$InsP_6ED_8$ (%)	45^{d}	46^{d}	54^{c}	72^{b}	78^{a}	79^{a}	0.9	<0.0001	<0.0001	0.007

Abbreviations: RSM = rapeseed meal; SBM = soybean meal; *a* = rapidly degradable amount, *b* = potentially degradable amount, *c* = degradation rate constant of *b*; $InsP_6ED_{2,5,8}$(mg) = effective degradation expressed as amount at rumen passage rates of 0.02, 0.05, and 0.08 h^{-1}; $InsP_6ED_{2,5,8}$(%) = percentage effective degradation of $InsP_6$ at rumen passage rates of 0.02, 0.05, and 0.08 h^{-1}.

[1] Incubation of the oilseed meals with $InsP_6$ amounts equal to 60, 120, and 180 mg (n = 3 replicates).

[a,b,c,d,e,f] Values within a row with different superscripts differ significantly at $P < 0.05$.

Table 4.3.3

Linear regression of effective degradation of phytate ($InsP_6$), expressed as amount, in response to increased $InsP_6$ amount.

	RSM				SBM			
	Slope[1]	Intercept	R^2	RMSE	Slope	Intercept	R^2	RMSE
$InsP_6ED_2$(mg)	0.83	−6.4	1.00	2.89	0.94	−2.7	1.00	0.74
$InsP_6ED_5$(mg)	0.67	−9.3	0.99	4.01	0.88	−5.2	1.00	1.55
$InsP_6ED_8$(mg)	0.58	−10.0	0.98	4.15	0.82	−6.3	1.00	1.96

Abbreviations: RSM = rapeseed meal; SBM = soybean meal; $InsP_6ED_{2,5,8}$(mg) = effective degradation expressed as amount at rumen passage rates of 0.02, 0.05, and 0.08 h^{-1}.
[1] Slopes and intercepts of regression lines for y ($InsP_6ED$(mg)) depending on x (60, 120, and 180 mg of $InsP_6$) were different from 1 and 0, respectively ($P < 0.05$) (n = 3 replicates).

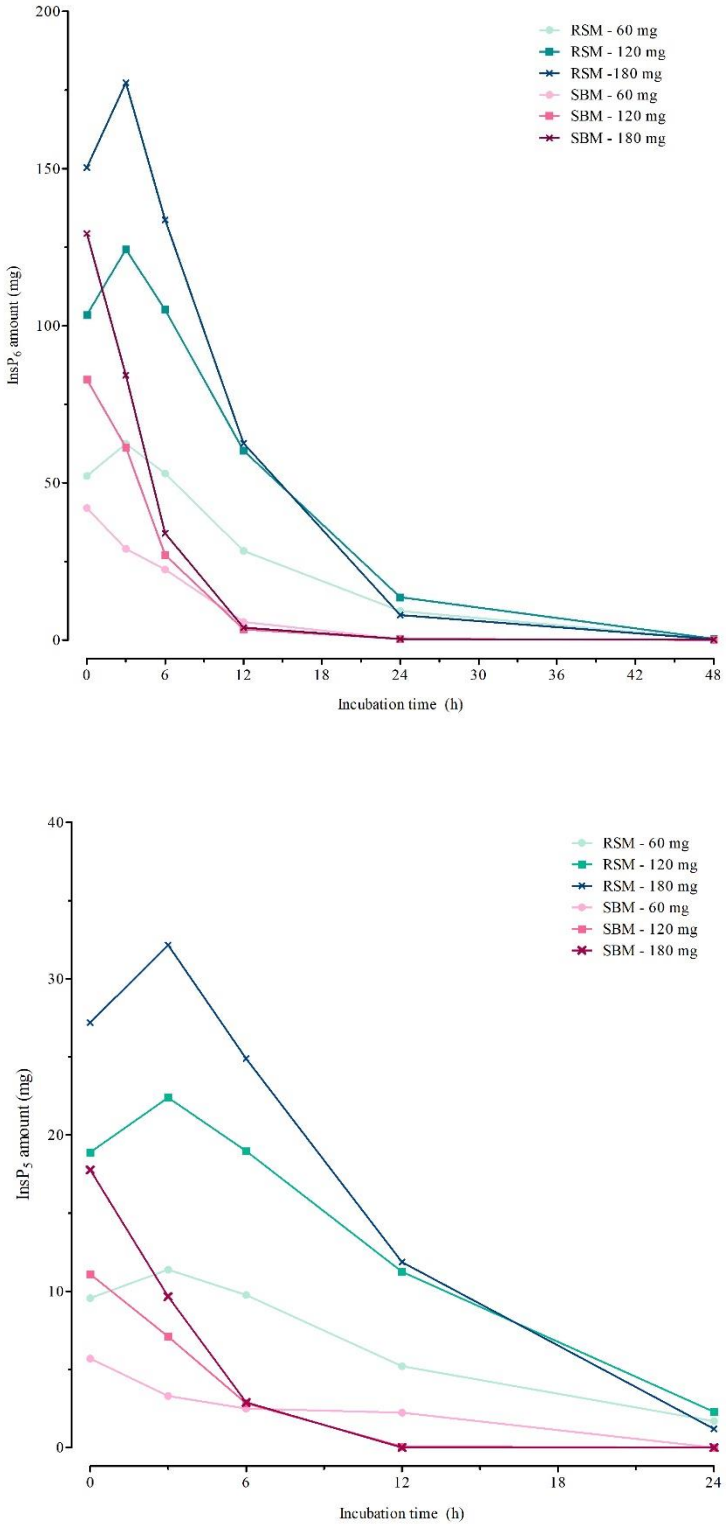

Figure 4.3.1

Amounts of inositol phosphates ($InsP_6$ and $InsP_5$) recovered from bag residues of rapeseed meal (RSM) and soybean meal (SBM) with 60, 120, and 180 mg of $InsP_6$ (RSM-60; RSM-120; RSM-180; SBM-60; SBM-120; SBM-180). Data are presented as the mean values of n = 3 replicates.

5 GENERAL DISCUSSION

The extensive discussion regarding ruminal and post-ruminal $InsP_6$ degradation, including the underlying reasons of the observation, are subject of this chapter. Besides, *in vivo*, *in situ*, and *in vitro* studies were used to evaluate ruminal degradation, and hence methodological aspects and their advantages as well as limitations are compared and discussed in detail in Chapter 5.1.

5.1 METHODOLOGICAL ASPECTS

Since ruminal $InsP_6$ degradation is considered to be important for $InsP_6$-P utilization in ruminants (Brask-Pedersen et al., 2013; Haese et al., 2014; Raun et al., 1956; Ray et al., 2013) and ruminal nutrient degradation is commonly assessed *in vivo*, *in situ*, and *in vitro*, one of the main intentions of the present thesis was to compare the results of ruminal degradation measured based on different methods (Figure 1).

Study of ruminal degradation based on an *in vivo* approach enables a direct estimation of nutrient digestion from animals which is often conducted by rumen evacuation (Bayat et al., 2007; Huhtanen et al., 2007; Krizsan et al., 2010), flow measurement combined with omasal (Ahvenjärvi et al., 2003; Foskolos et al., 2020), abomasal (Westreicher-Kristen et al., 2018), or duodenal sampling (Ahvenjärvi et al., 2000), and digesta sampling from abomasum subsequent to sacrifice of animals (Park et al., 2002). Data obtained *in vivo* are the most reliable owing to the actual response of animals to the dietary treatments, but the processes are laborious, time-consuming, and costly, and the numbers of animal used for treatments are restricted. While harvesting representative digesta samples posterior to the rumen is decisive for the accurate measurement of *in vivo* rumen digestion, the most appropriate sampling method remains controversial, because particle passage kinetics (Ahvenjärvi et al., 2004; Bayat et al., 2011), marker choice (Ahvenjärvi et al., 2003; Faichney et al., 1989), and sampling site (Ahvenjärvi et al., 2000; Fatehi et al., 2015) as well as sampling devices (Westreicher-Kristen and Susenbeth, 2017) can greatly influence the results of rumen digestion. In Manuscript 1, TiO_2 was used as indigestible marker and its concentration in omasum combined with abomasum (omasum + abomasum) was calculated for *in vivo* ruminal $InsP_6$ disappearance. It was assumed that TiO_2 concentration has reached

the steady state in the animal at the time of sampling (after 5 days of oral administration) as suggested by Owens and Hanson (1992). However, due to the limited knowledge, it is not known to which extent TiO_2 is reliable for evaluating rumen digestion. Although Myers et al. (2006) examined the duodenal concentration of TiO_2 changing over time in ewes and stated that TiO_2 may be suitable for the determination of rumen digestion based on duodenal sampling, the recovery of TiO_2 in duodenum was not measured in this study. In cattle, a recovery rate of TiO_2 higher than 90% has been reported based on the measurement of the faecal samples, indicating that TiO_2 is an appropriate marker to estimate total tract nutrient digestion (Titgemeyer et al., 2001). Nevertheless, digesta composition and particle distribution differ along the digestive tract, with a more heterogeneous digesta in omasum and abomasum compared to large intestine. It is likely that a combination of several phase-associated markers provides a more accurate assessment of rumen digestion (Faichney, 1980; Faichney et al., 1989), compared to using TiO_2 as the single marker. In addition, *in vivo* results are generally prone to variation of animals. The rumen conditions of individual animals can differ markedly in rumen volume, pH, temperature, digesta passage, and microbial profile, all of which may exert effects on rumen digestion. In Manuscript 1, the individual difference between dietary treatments was of study interest, and hence we did not pool the values of the harvested samples. A variation of greater than 20% in ruminal $InsP_6$ disappearance was observed among animals fed a diet containing RSM. This will be discussed in details in chapter 5.4.

In comparison with *in vivo* sampling techniques, the *in situ* approach allows a simpler and less intensive determination of rumen digestion. *In situ* study is commonly conducted by weighing a certain amount of feed in nylon or polyester bags and then placing them in the rumen for a certain time period. Despite the direct incubation and characterization of feed, *in situ* results are susceptible to methods adopted by the laboratory and can be influenced by sample size (Diao et al., 2020; Nocek, 1985), feed grinding (Damiran et al., 2008), bag material and pore size (Nocek, 1985; Valente et al., 2011), animal feeding (Schadt et al., 2014), bag rinsing method (Cherney et al., 1990), incubation time (Olaisen et al., 2003), etc. In an attempt to increase the comparability and reproducibility, recommendations for a standardised *in situ* procedure have been proposed (Vanzant et al. 1998; NRC 2001; GfE 2022). Nonetheless, the *in situ* approach has its limitations and is often criticised about the

uncertainty whether small particles escaping bags are completely degraded or whether the microbial attachment can be entirely removed by bag rinsing (Olubobokun et al., 1990; Seifried et al., 2015). Besides, feeds are confined in the bags without being subjected to mastication and rumination. The cumulative nutrient disappearance over course of the *in situ* incubation is subsequently used for the calculation of rumen effective degradation, and these values are highly dependable of criteria and constraints that are chosen for the model (Ørskov and McDonald, 1979; Wulf and Südekum, 2005). Thus, results obtained from *in situ* technique should be interpreted with caution. In Manuscript 1 and Manuscript 2, the rumen incubation and choice of animals were conducted according to the recommendation established by GfE (2022), and the calculation of $InsP_6ED$ was based on Ørskov and McDonald (1979), McDonald (1981), and Wulf and Südekum (2005). The calculated $InsP_6ED_5$ in Manuscript 1 was 64% for RSM and 85% for SBM. These results agree with the results from previous studies that $InsP_6ED$ for RSM is lower than that for SBM (Haese et al., 2020; Konishi et al., 1999; Park et al., 2000), and the data are similar to the values of $InsP_6ED_5$ reported by the authors, which was 59%, 68%, and 69% for RSM and 76%, 81%, and 81% for SBM in the study by Haese et al. (2020), Konishi et al. (1999), and Park et al. (2000), respectively. The variation between studies may be partly attributed to the methodical differences between the experiments, as discussed above. However, even when conducted with the same *in situ* procedures, a considerable difference of $InsP_6ED$ was observed among SBM samples (Table 1). The values of $InsP_6ED$ differed not only between *in situ* studies but also between samples in the same study, indicating that a general value for a certain feedstuff to assess rumen $InsP_6$-P digestion may not be precise enough.

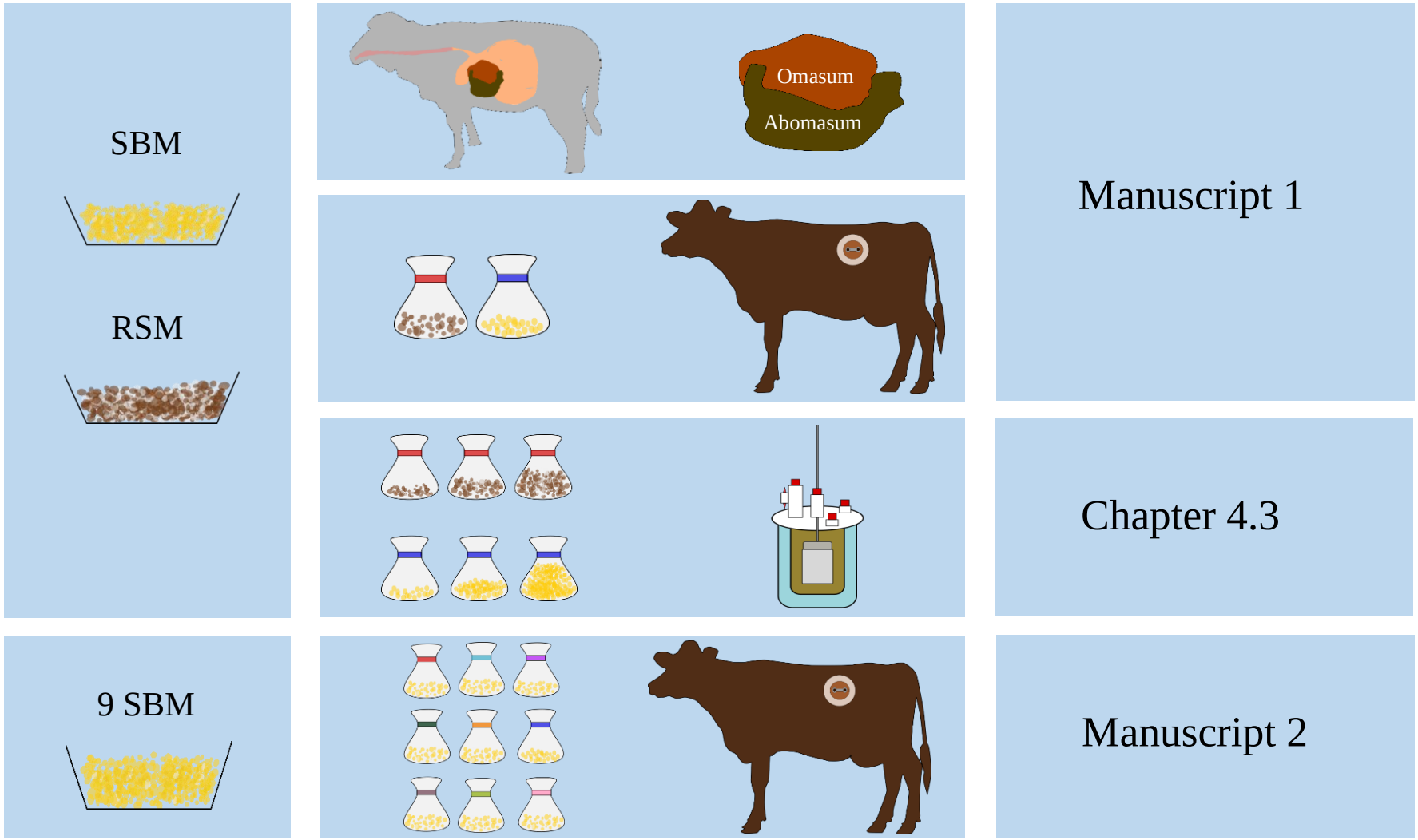

Figure 1. Illustration of used methods (*in vivo*, *in situ*, and *in vitro*) to study ruminal $InsP_6$ degradation in this doctoral thesis. The same batches of rapeseed meal (RSM) and soybean meal (SBM) were used in Manuscript 1 (*in vivo* and *in situ*) and in Chapter 4.3 (*in vitro*). Nine SBM from different origins were used in Manuscript 2 (*in situ*).

Table 1. *In situ* calculated rumen effective degradation of phytate ($InsP_6ED$, %) at different rumen passage rates for 9 soybean meal (SBM) variants.[*]

	SBM										
No.[#]	2	4	5	9	10	11	12	14	18	SEM	*P*-value
$InsP_6ED_2$[§]	94[a]	89[b]	93[a]	92[a]	93[a]	81[d]	84[c]	93[a]	88[bc]	0.66	<.0001
$InsP_6ED_5$	86[a]	79[c]	84[ab]	83[b]	84[ab]	65[f]	69[e]	85[ab]	75[d]	1.28	<.0001
$InsP_6ED_8$	81[a]	71[c]	77[b]	76[b]	77[b]	55[f]	59[e]	79[ab]	66[d]	1.64	<.0001

[*]Data are presented as least square means (n = 3 cows) with standard error of the means (SEM).
[#]Numbers of soybean meal variants are identical to Manuscript 2.
[§]$InsP_6ED$ was calculated for rumen passage rates of 0.02 ($InsP_6ED_2$), 0.05 ($InsP_6ED_5$), and 0.08 ($InsP_6ED_8$) h^{-1}.
[a–f]Different superscripts indicate significant differences within a row ($P < 0.05$).

In contrast to *in vivo* and *in situ* studies, *in vitro* incubation allows a simultaneous assay of feed samples and combinations of treatment factors at a larger scale. *In vitro* model intends to simulate the *in vivo* rumen digestion which requires rumen fluid obtained from animals and buffer solution that mimics the salivation of ruminants. The greatest advantage of this study approach is its relatively low cost and convenience. *In vitro* study of rumen digestion can be carried out with batch culture that is mainly used for short-period measurement of digestion kinetics, or with the RUSITEC which enables a long-period estimation of rumen fermentation and microbial metabolism (Boguhn et al., 2006; Haese et al., 2014; Künzel et al., 2022). Nevertheless, a primary reference for standardised *in vitro* procedure is not available. *In vitro* methods vary among laboratories in ratio of rumen fluid to buffer solution, type of vessels, incubated sample amount, sample grinding, diet of rumen fluid donor animal, sampling technique, and so on, all of which can cause deviations between studies and render the comparison of data among different studies difficult (Boguhn et al., 2013; Camacho et al., 2022; Dennison and Marais, 1980; Haese et al., 2017a; Komisarczuk et al., 1987). To minimise systematic errors and increase repeatability as well as reproducibility among laboratories, Camacho et al. (2022) suggested using a standard procedure for *in vitro* experiment with rumen fermenters. By following the *in vitro* method proposed by

Brazilian National Institute of Science and Technology in Animal Science, the *in vitro* DM digestibility for feeds showed a lower random variation among laboratories than within laboratories (Camacho et al., 2022). This supports the necessity of a standardised *in vitro* manual for studying rumen digestion. However, adjustments may be required when different nutrients or chemicals are to be studied. Aside from the variation among laboratories, one major concern of using *in vitro* approaches exists in that it may not represent the actual rumen environment due to a possible alteration in microorganisms and dilution of rumen fluid, and these can affect the rumen digestion (Brask-Pedersen et al., 2011; Morse et al., 1992). In Chapter 4.3, despite the similar pH of buffer solution and ruminant saliva (pH 8.2–8.6), the solution was prepared without containing phosphate, because inorganic P has been reported to decrease microbial phytase efficiency (Godoy and Meschy, 2001; Haese et al., 2014). Inorganic P, however, has been suggested to be one of the substantial components in the saliva of sheep (McDougall, 1948) and cattle (Bailey, 1961), and the concentration of inorganic P in saliva can vary with different factors, such as diet composition (Bailey, 1961), dietary P concentration (Valk et al., 2002), and plasma phosphate concentration (Mañas-Almendros et al., 1982). Based on these concerns, it is advisable to compare the results obtained by applying different techniques and assess the discrepancies therebetween in order to evaluate the quality of results and reliability of the findings.

As described in Manuscript 1, the wethers were assumed to have a low rumen passage rate, because they were fed at maintenance energy requirement level. For wethers fed a diet containing RSM, ruminal $InsP_6$ disappearance was measured to be 76%, and that was 89% for wethers fed a diet containing SBM. These values are similar to the *in situ* calculated $InsP_6ED_2$ in the same manuscript, which was 83 and 93% for RSM and SBM, respectively. In Chapter 4.3, the equation proposed by McDonald (1981) was used in an attempt to evaluate *in vitro* calculation of $InsP_6ED$ for the same batches of RSM and SBM, and the averaged value of $InsP_6ED_2$ (over the three incubated amounts of $InsP_6$) was 77% for RSM and 92% for SBM. Compared with the *in situ* calculated $InsP_6ED$, the *in vitro* calculated values were even more close to the ruminal $InsP_6$ disappearance determined *in vivo*. Besides, the averaged $InsP_6ED_5$ was 83% for SBM and 59% for RSM in Chapter 4.3, with a difference about 20% therebetween. This is consistent to the previous *in situ* results from the Department of Animal Nutrition at University of Hohenheim, which were calculated for different batches of RSM and SBM (e.g.

$InsP_6ED_5$: 76% for SBM and 59% for RSM in the study by Haese et al. (2020)). Thus, an estimation of ruminal $InsP_6$ degradation based on *in vitro* method seems to be possible. However, more studies are required for the validation and calibration.

In addition to the similar ruminal $InsP_6$ degradation measured by different methods, a rapid degradation of solubilised $InsP_6$ was also observed in Manuscript 1 and Chapter 4.3. In Manuscript 1, $InsP_6$ concentration was below the detection limit (< 0.13 g/kg DM) in the pulverised fluid phase samples of rumen content; in Chapter 4.3, $InsP_6$ was not detectable (< 33 mg/l) in the liquid samples of rumen fluid/buffer mixture, and fluid in the fermenter or beaker at the targeted incubation period. As discussed in Chapter 4.3, this is consistent to the previous study by Haese et al. (2017), where only very low concentrations of $InsP_6$ were determined in the rumen fluid/buffer mixture before and after the incubation (< 20 mg/l for high P diet). These observations are highly relevant for *in vitro* and *in situ* experiments using bags for incubation because it is commonly questioned whether the nutrients escape the bags are degradable. In regard to $InsP_6$, the data consistently indicate that material escaping the bag is degraded to very large extent. Moreover, only traces of $InsP_5$ but no $InsP_{1–4}$ was detected in the particulate phases of rumen content in Manuscript 1, and no accumulation of $InsP_{3–5}$ was observed throughout the *in situ* and *in vitro* incubation in Manuscript 2 and Chapter 4.3, respectively. This not only shows a great consistency of the results in ruminal $InsP_6$ degradation among studies using different methods but also agrees with the findings by Brask-Pedersen et al. (2011) and Haese et al. (2020) that cleavage of the first phosphate group from $InsP_6$ is the decisive step of $InsP_6$ degradation.

5.2 RUMINAL $INSP_6$ DEGRADATION

In Manuscript 1, *in situ* calculated $InsP_6ED$ values were significantly lower for RSM compared to SBM and a lower ruminal disappearance was also observed when RSM was included in the diet fed to wethers. In Manuscript 2, the examined 9 SBM variants varied markedly in *in situ* degradation parameters and hence the calculated $InsP_6ED$ differed significantly among the SBM variants (61–84% at a rumen passage rate of 0.06 h^{-1}), and $InsP_6ED$ were significantly correlated to CPED as well as CNCPS protein fractions (Table 2). In Chapter 4.3, *in vitro* degradation kinetics differed not only between RSM and SBM but also among different $InsP_6$ amounts of these two oilseed

meals, with significant interaction effect of oilseed meal × $InsP_6$ amount for calculated $InsP_6ED$. The underlying reasons of the observed effects and the causes of variations are discussed as following:

Table 2. Pearson correlation coefficients between phytate ($InsP_6$) degradation and crude protein (CP) degradation and protein fractions ($P < 0.05$).*

	In situ CP degradation (%)	CNCPS protein fractions† (% of CP)				
	$CPED_6$§	A	B_1	B_2	B_3	C
$InsP_6ED_6$# (%)	0.88	−0.91	0.69	0.70	−0.85	−0.91

*Significance of the parameters was also determined for rumen passage rates of 0.05 and 0.08 h^{-1}

#$InsP_6ED_6$, rumen effective degradation of $InsP_6$ at a rumen passage rate of 0.06 h^{-1}.

§$CPED_6$, rumen effective degradation of $InsP_6$ at a rumen passage rate of 0.06 h^{-1}.

†Protein fractions were determined based on Cornell Net Carbohydrate and Protein System (CNCPS).

5.2.1 LOCALIZATION AND BINDING FORM OF $INSP_6$ IN OILSEEDS

Rapeseed and soybean are dicotyledonous seeds, in which $InsP_6$ accumulates and condenses during seed ripening inside of a protein-rich organelle, specified as protein storage vacuole (PSV) (Gillespie et al., 2005; Prattley and Stanley, 1982). The PSV contains matrix and membranous compartments (e.g. globoid and crystalloid) where storage proteins, $InsP_6$, lipids, and minerals are located (Figure 2) (Isayenkov, 2014; Jiang et al., 2001). However, distinctions exist in chemical distribution and composition between oilseeds, which can influence the solubility and accessibility of $InsP_6$, and consequently the extent of $InsP_6$ degradation.

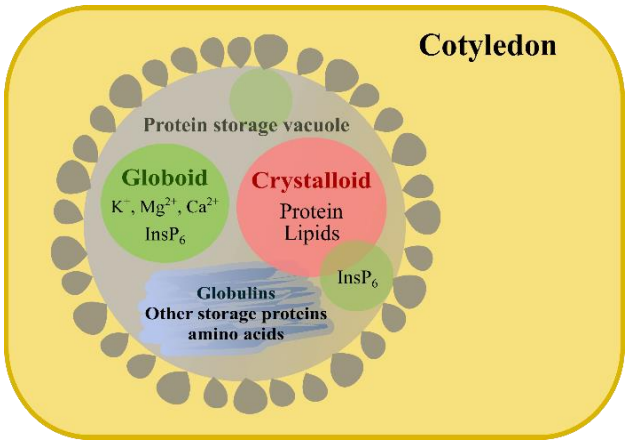

Figure 2. Illustration of common oilseed protein storage vacuole.

Differing from most of other oilseeds, soybean has only a small fraction of $InsP_6$ included in the globoids (0.1–1.0 μm in diameter), but most of $InsP_6$ is uniformly distributed in the matrix of PSV (Lott and Buttrose, 1978; Prattley and Stanley, 1982). The $InsP_6$ in rapeseed, by contrast, was found to be predominantly confined in numerous globoids (0.5–2.8 μm in diameter) that are bound to crystalloids within the PSV (Gillespie et al., 2005; Yiu et al., 1982). The additional membranous structure and the connection of globoid to crystalloid in PSV may render the degradation of other cell components necessary before microbial phytases can approach $InsP_6$, delaying the degradation process of $InsP_6$. The lower accessibility of rapeseed $InsP_6$ is likely to explain the observation in Manuscript 1, where a lag phase of 3.6 h for RSM and 0.99 h for SBM was determined ($P < 0.05$). Similarly, Haese et al. (2017b) observed a delayed $InsP_6$ disappearance for RSM compared to SBM, with approximately 30% of $InsP_6$ disappearance already after 2 h of incubation for SBM but not for RSM. However, the accessibility of $InsP_6$ seems to be increased by structural changes when RSM is treated with heat, and hence a higher $InsP_6$ disappearance occurs in the earlier incubation hours (10% and 18% after 2 and 4 h of incubation, respectively), as reported by Haese et al. (2017b).

Owing to the polyanionic potential of $InsP_6$ molecule by carrying 12 dissociable protons, $InsP_6$ can react with other seed components and chemicals in the PSV under different conditions (Hídvégi and Lásztity, 2002; Kies et al., 2006; Selle et al., 2012). This includes, for example, a strong tendency of negatively charged $InsP_6$ to chelate the cations of metal elements (mainly Ca, Mg, and K) in the PSV of oilseeds, forming

cation-phytate complexes (Kolláthová et al., 2019; Lott et al., 1985). However, the ratio of these metal elements varies between seed types (Kolláthová et al., 2019; Lott et al., 1985), and hence the size of cation-phytate complexes and the solubility thereof differ among oilseeds (Lott et al., 1985; Prattley and Stanley, 1982). For soybean, the content of K, Mg, and Ca was determined to be at a ratio of about 14:2:1 (Prattley and Stanley, 1982), and $InsP_6$ was present predominantly in the form of K-phytate (Lott et al., 1985). In contrast to soybean, rapeseed contains a considerably higher amount of Mg- and Ca-phytate in the PSV (Gillberg and Törnell, 1976). With the increased bonding between metal ions and $InsP_6$, solubility of the cation-phytate was found to decrease (Sun et al., 2021). Previous studies have reported that K-phytate is generally soluble (Cheryan, 1980), whilst solubility significantly reduces at pH > 4 for Ca-phytate (Grynspan and Cheryan, 1983) and at pH > 5 for Mg-phytate (Cheryan et al., 1983). The higher proportion of K-phytate in soybean may partly explain the higher *a* values of $InsP_6$ for SBM than for RSM determined *in situ* in Manuscript 1 (32 vs. 1.9%) and *in vitro* in Chapter 4.3 (33 vs. 13%, expressed as averaged values over all 3 treatment amounts of $InsP_6$), which is consistent to the level of values (27% vs. 0%) reported by Haese et al. (2020). Solubility of $InsP_6$ is decisive for $InsP_6$ degradation because unsolubolised $InsP_6$ can also lower the accessibility of microbial phytases to their targeted P-O bond of $InsP_6$, and hence slow down the degradation of $InsP_6$ (Sun et al., 2021).

5.2.2 INTERACTION OF $INSP_6$ WITH CRUDE PROTEIN AND RELATION OF $INSP_6$ DEGRADATION TO CRUDE PROTEIN DEGRADATION

In addition to the chelation between $InsP_6$ and cations, the $InsP_6$ molecule has a high capacity to interact with storage protein and form binary protein-phytate complexes or ternary protein-cation-phytate complexes as proteins are also charged when the pH is not equalised to their isoelectric point (Reddy and Salunkhe, 1981; Selle et al., 2012). The isoelectric point for soybean protein is around pH 4.8 (Feng et al 2015), while that for rapeseed protein seems to vary widely between pH 4–11 due to the complicated protein composition in rapeseed (20−40% close to pH 11; others between pH 4 and 8) (Gillberg and Törnell, 1976; Lönnerdal et al., 1977). At pH lower than the isoelectric point, proteins carry a net positive charge and forms binary protein-phytate complexes with negatively charged $InsP_6$ molecules (Reddy and Salunkhe, 1981). On the contrary,

proteins carry a net negative charge at pH higher than their isoelectric point and form ternary complexes through a cationic bridge that links $InsP_6$ and protein molecule together (Reddy and Salunkhe, 1981). This contributes to the tight association between $InsP_6$ and protein molecules (Selle et al., 2012), as previously suggested for soybean (Brooks and Morr, 1982) and for rapeseed (Gillberg and Törnell, 1976; Gillespie et al., 2005). A high coefficients of determination (R^2) (> 0.90) calculated by regression analysis of $InsP_6$ disappearance based on CP disappearance (% at targeted h) for RSM and SBM also supports the existence of protein-phytate complexes in oilseeds (Haese et al., 2017b). However, interactions between $InsP_6$ and CP and solubility of protein-phytate complexes have been shown to be influenced by various factors (Amat et al., 2022; Kies et al., 2006; Selle et al., 2012). For instance, the naturally occurred interaction between $InsP_6$ and CP in feed was investigated *in vitro* by Kies et al. (2006), who found that additional $InsP_6$ favors the further $InsP_6$ agglomeration with CP at certain pH and reduces the solubility of protein-phytate complexes. For RSM and SBM used in Manuscript 1 and Chapter 4.3, the ratio of $InsP_6$ to CP was approximately 0.06 for RSM and 0.03 for SBM, which renders the possibility high that the binding of $InsP_6$ and CP is more saturated and the interaction therebetween is more intense in RSM than in SBM. Moreover, as discussed in Chapter 5.2.1, rapeseed contains a higher fraction of Ca^{2+} and Mg^{2+} than K^+ in the PSV. Connection of CP and $InsP_6$ tends to be stronger in the presence of polyvalent cations due to the elevated ionic strength (Hídvégi and Lásztity, 2002). These might explain the discordant relationship observed between the examined RSM and SBM in Manuscript 1, with an almost identical $InsP_6$ED and CPED for RSM against a difference of approximately 20 percentage points between $InsP_6$ED and CPED for SBM. A similar pattern can also be found in the studies by Konishi et al. (1999) and Park et al. (1999), where $InsP_6$ED and CPED resemble each other for untreated RSM, but these are calculated to differ greater than 30 percentage point from each other for untreated SBM.

The linkage between CP and $InsP_6$ remains, and the molecular interaction therebetween may be reinforced by processing of oilseeds (Wang et al., 2018; Yiu et al., 1983), during which high pressure and temperature are often applied, in order to minimise deterioration and maintain quality of feed, or to remove anti-nutrients. However, these processing conditions can induce cross-linking of AA and peptides that react with carbonyl groups on reducing sugars, forming condensed protein-sugar aggregates

through Maillard reaction (Lan et al., 2010). Maillard-induced protein denaturation has been reported to lower solubility and degradability of CP in the rumen (Little et al., 1963), and change digestibility of other associated nutrients (Chung et al., 2012). Therefore, if processing conditions applied for the production of oilseed meals differ among oil processing plants, which affect CP degradation (Steingass et al., 2013), it is possible that $InsP_6$ is also affected due to the tight association between $InsP_6$ and CP (Fontaine et al., 1946; Saio et al., 1967; Yiu et al., 1983). This is supported by the previous *in situ* study by Konishi et al. (1999), who observed a significant reduction of ruminal CP degradation paralleled with a reduction of ruminal $InsP_6$-P degradation upon heating. The authors suggested that heat treatment reduced solubility of protein-phytate complexes in the rumen which led to the suppression of ruminal $InsP_6$-P degradation (Konishi et al., 1999). However, the effect of heating on ruminal $InsP_6$-P degradation seems to be more intensive for RSM, as a greater reduction in ruminal $InsP_6$-P degradation for RSM than for SBM was observed with temperature increasing from 133 to 153ºC (around 40% and 20% for RSM and SBM, respectively) (Konishi et al., 1999). The difference in effect of heating might be attributed to the distribution of $InsP_6$ in the PSV (Konishi et al., 1999) and other factors that can affect interaction between $InsP_6$ and CP ($InsP_6$ to CP ratio and presence of polyvalent cations, as discussed before). Also, rapeseed storage protein is mainly consisted of cruciferin, which has a lower thermostability compared to soybean glycinin and this feature of protein favors the formation of insoluble aggregates upon heating (Mohamad Ramlan et al., 2002).

The close relationship between $InsP_6$ and CP degradation and the intense influence of processing on ruminal degradation for RSM was also confirmed in a recent *in situ* study by Haese et al. (2022). By prolonging the residence of desolventising/toasting, protein composition of RSM was found to shift from rapidly and intermediate degradable protein fractions (B_1 and B_2) to slowly degradable and unavailable protein fractions (B_3 and C), which was reflected in the CPED values, and $InsP_6$ degradation was found to be affected almost identically to CP degradation (Haese et al., 2022). A highly significant correlation was determined between $InsP_6ED_5$ and $CPED_5$ (r = 0.97) for 9 rapeseed feeds and between $InsP_6ED_5$ and CNCPS protein fractions B_1 (r = 0.84), B_2 (r = 0.97), and C (r = −0.94) for 6 differently processed RSM samples. For SBM samples analysed in Manuscript 2, it was not possible to evaluate the effect of processing, since

only the information about the countries where the commercial SBM samples came from was available. However, SBM samples varied in a similar pattern in *in situ* parameters of CP and $InsP_6$ degradation, and a close relationship between $InsP_6$ and CP degradation was also identified for SBM from different origins (see Table 2). As discussed in Manuscript 2, the significant correlations between $InsP_6$ and CP degradation for RSM and SBM could be useful for predicting the $InsP_6$ degradation based on CP degradation, yet it seems that a general equation for different feedstuffs is not possible and feedstuff-specific equations may be required. Overall, it was not easy to compare the differences between RSM and SBM and to draw a precise conclusion from Manuscript 2 and the study by Haese et al. (2022) because of the limited sample size and differed backgrounds such as processing methods and agronomic conditions of the examined feeds. Besides, most of the SBM samples from Manuscript 2 tend to have high values of CP degradation ($CPED_6$ > 55%) which renders the bias of correlation test high. To apply the results in practice and to validate the prediction models, it is recommended to analyse samples at a larger numbers with different origins and a wide range of CP degradability.

The association between $InsP_6$ and CP might account for the negative values of $InsP_6$ disappearance or increased $InsP_6$ amount determined from bag residues of RSM at 2 h in Manuscript 1 (e.g. $InsP_6$ disappearance: −2.4%) and at 3 h in Chapter 4.3 (e.g. $InsP_6$ disappearance: −4.7% for 60 mg and 120 mg of $InsP_6$), and the higher $InsP_6$ concentrations determined in the 0 h bag residues compared to those determined in feeds without incubation for both RSM and SBM (observed in Manuscript 1, Manuscript 2, and Chapter 4.3) (Table 3). As discussed in Chapter 4.3, the findings are similar to the results from previous *in situ* (Haese et al., 2017b) and *in vitro* studies (Haese et al., 2017a), and the underlying reason is probably that a part of $InsP_6$ bound in insoluble protein-phytate complexes was not accessible for microbial phytases and extractants before protein polymers underwent structural changes during incubation (Xin and Yu, 2013). However, SBM has a less intense association between $InsP_6$ and CP as described before, a higher fraction of soluble $InsP_6$ as observed in Manuscript 1 and Chapter 4.3, and more easily degradable feed constituents as indicated by the rapidly reduced DM, which made the counteracting degradation kinetics less pronounced in comparison to RSM (SBM: with reduced $InsP_6$ concentration already after 2 and 3 h of incubation in Manuscript 1 and Chapter 4.3). In the *in vitro* study by

Kies et al. (2006), the recovery of $InsP_6$ was observed to be low in extracts of sunflower seed meal and RSM but high in maize followed by SBM. The authors ascribed the lower recovery of $InsP_6$ to the small amount of soluble protein-phytate complexes in the feed. Combined with the results from the present and other studies (Haese et al., 2017a, 2017b; Kies et al., 2006), it appears that $InsP_6$ determination from feed and bag residues of earlier incubation hours is highly dependent on CP solubility and degradation for oilseed-derived feedstuffs. Therefore, to allow a more precise calculation of $InsP_6$ degradation of feeds, further research is necessary to find a more suitable method for extraction of $InsP_6$, potentially with the combination of protease or with a different pH of the buffer solution, since these can influence protein solubility and degradation (Kies et al., 2006).

5.2.3 INSP$_6$ AMOUNT

As mentioned in Manuscript 1, due to the higher intake of $InsP_6$ from the diet containing RSM (5.9 g/d) compared to the diet containing SBM (3.8 g/d), ruminally degraded amount of $InsP_6$ was calculated to be higher in wethers fed a diet containing RSM (4.5 g/d vs. 3.4 g/d), and this is consistent with the findings by Ray et al. (2013) that dairy cows fed higher amounts of $InsP_6$-P also showed a greater ruminal $InsP_6$-P degradation. As suggested by these authors, increased ruminal $InsP_6$ degradation may be attributed to the enhanced phytase activity of rumen microorganisms, through their utilization of the substrate for growth and function (Godoy and Meschy, 2001; Yanke et al., 1998). However, Manuscript 1 used either RSM or SBM as $InsP_6$ source and Ray et al. (2013) achieved low, medium, and high levels of dietary $InsP_6$-P by combining cotton seed meal with SBM at different ratios (0/14, 13/7, and 25/0, respectively). Both of the studies (Manuscript 1 and the study by Ray et al. (2013)) interpreted the effect of $InsP_6$ amount based on different $InsP_6$ sources without also considering the amounts. Such conclusions may be biased because feeds differ in their degradation kinetics (Haese et al., 2020, 2017b; Morse et al., 1992) which can influence ruminal degradation. As outlined in the *in vitro* study (Chapter 4.3), different patterns of degradation parameters were determined between RSM and SBM in response to increased $InsP_6$ amount, and the calculated $InsP_6$ED expressed as amount ($InsP_6$ED(mg)) increased to a greater extent for SBM than for RSM. When incubating SBM with 120 mg $InsP_6$, the calculated

$InsP_6ED$(mg) at a rumen passage rate of 0.08 h^{-1} was almost the same as when incubating RSM with 180 mg $InsP_6$ (93 and 96 mg, respectively; $P > 0.05$).

In addition to the different $InsP_6$ source that can influence the interpretation of effect of $InsP_6$ amount, the way to express degradation can also affect the observed effects. As mentioned in Chapter 3, the ruminal disappearance was lower for wethers fed a diet containing RSM than those fed a diet containing SBM, but a higher amount of $InsP_6$ was ruminally degraded in wethers fed a diet containing RSM. In Chapter 4.3, $InsP_6ED$ expressed in percentage ($InsP_6ED$(%)) increased with increasing $InsP_6$ amount from 120 to 180 mg for RSM and from 60 to 120 mg without further increase up to 180 mg for SBM, despite the linear increase in $InsP_6ED$(mg) with increasing $InsP_6$ amount for both RSM and SBM. In fact, a more rapid decline and a higher amount of degraded $InsP_6$ were observed upon increasing $InsP_6$ amount over the course of incubation, and hence relative values may not have reflected the real dynamics of quantitative $InsP_6$ degradation.

It should be kept in mind that aside from the aforementioned aspects which can influence the observed effect of $InsP_6$ amount, other experimental conditions might also contribute to difference in results of ruminal $InsP_6$ degradation, causing variation between studies. For example, Haese et al. (2014) compared $InsP_6$ disappearance between control diet and high $InsP_6$ diet from cows (*in vivo*) and from the RUSITEC system (*in vitro*) and found the opposite effects of the diets (*in vivo*: high $InsP_6$ diet > control diet; *in vitro*: high $InsP_6$ diet < control diet). The lower $InsP_6$ disappearance determined *in vitro* for the high $InsP_6$ diet compared to the control diet was attributed to the low concentrate diet fed to the donor animals of rumen fluid for incubation as this favors the growth of cellulolytic bacteria in the system, whereas phytase activity has only been determined from amylolytic bacteria so far (Carrizo et al., 2016; Songré-Ouattara et al., 2008; Yanke et al., 1998). This implies that effect of $InsP_6$ amount can be influenced by the diet composition of animals due to the shift of microbial population in response to the diet, and hence animals used in *in vivo* and *in situ* studies or animals used to obtain rumen fluid for *in vitro* study should be adapted to the diet similar to the experimental diet. On the other hand, Godoy and Meschy (2001) did not observe difference in *in vitro* $InsP_6$ disappearance between treatments of $InsP_6$-P amount although the animals used for inoculum were adapted to the high or low $InsP_6$-P diet. In their study, buffer P was supplied either with inorganic P as monosodium phosphate,

or with organic P as maize Na-phytate. However, high concentration of inorganic P has been reported to lower the phytase activity (Haese et al., 2017b, 2014), and maize Na-phytate is highly soluble and rapidly degradable compared to Ca- or Mg-phytate in oilseeds as discussed in Chapter 5.2.1. This suggests that the composition of the buffer solution used for *in vitro* study is also relevant for the effect of $InsP_6$. As discussed in Chapter 4.3, the respective amount of $InsP_6$ was achieved by incubating different amounts of single feed, and hence the amount of other nutrients such as nitrogen and carbohydrates also differed among treatment groups. This means that besides $InsP_6$, the additional nutrient and energy sources might also have favored the growth and function of certain phytase-producing microorganisms (Lan et al., 2002a). Moreover, the influence of slight pH change (between pH 6 and 7) due to different amounts of incubated feed in the *in vitro* system (i.e. reduction of pH in the fermenter fluids after incubation increased with the total DM amount of incubated feed) on microbial phytase activity could not be ruled out in this study (Haros et al., 2005; Lamid et al., 2018). Therefore, comparison of $InsP_6$ amount should be made with caution.

Table 3. Mean concentrations of phytate ($InsP_6$, μmol/g) analysed for rapeseed meal (RSM) and soybean meal (SBM) in different studies.[*]

	In situ (Manuscript 1)	
	RSM	SBM
feed	29.7	19.1
0 h	39.9	19.3

	In situ (Manuscript 2)								
	SBM[#]								
	2	4	5	9	10	11	12	14	18
feed	21.9	18.2	21.0	17.5	17.9	17.4	19.9	20.4	19.9
0 h	20.0	19.7	24.3	19.8	18.4	19.8	21.7	22.8	23.0

	In vitro (Chapter 4.3)					
	RSM			SBM		
	60 mg[§]	120 mg	180 mg	60 mg	120 mg	180 mg
feed	29.9	29.9	29.9	16.9	16.9	16.9
0 h	39.2	39.3	37.9	22.8	23.6	23.5

[*]Data are presented with n = 3 cows (*in situ)* or incubation vessels (*in vitro*).
[#]Numbers of soybean meal variants are identical to Manuscript 2.
[§]$InsP_6$ amounts equal to 60, 120, and 180 mg.

5.3 POST-RUMINAL $INSP_6$ DEGRADATION

Previously, Park et al. (2002) fed sheep with diets including 20% RSM and found a decreased passage of $InsP_6$ between the small intestine and the large intestine. In that study, 17% of dietary $InsP_6$ disappeared post-ruminally (between the abomasum and rectum) in the sheep, with 88% of post-ruminal $InsP_6$ degradation observed between the jejunum and colon. The authors attributed the post-ruminal $InsP_6$ degradation to microbial phytases in the upper large intestine. In contrast to the result reported by Park et al. (2002), the present study found that only 6% of dietary $InsP_6$ disappeared post-ruminally (between the omasum + abomasum and rectum) in wethers fed a diet containing RSM, which did not differ significantly from 4% determined in wethers fed a diet containing SBM (Manuscript 1). Calculating the data from the present study to the proportion of ruminal $InsP_6$ outflow, 27 and 35% of $InsP_6$ leaving the rumen was degraded post-ruminally in wethers fed a diet containing RSM and SBM, respectively. Similar to the present findings, post-ruminal $InsP_6$-P disappearance (between the duodenum and feces) in the study by Brask-Pedersen et al. (2013) was 4% in the cows fed a diet containing 20% of rapeseed cake on a DM basis, which is equivalent to 29% as proportion of ruminal $InsP_6$-P outflow. As discussed in Manuscript 1, the higher extent of post-ruminal $InsP_6$ degradation observed by Park et al. (2002) may have resulted from the higher fraction of potentially rumen degradable $InsP_6$ in the feed that escaped the rumen degradation owing to the insufficient retention time of digesta. In the present study, the digestion time for $InsP_6$ in the rumen of wethers was assumed to be long enough due to the low feeding level, and thus, $InsP_6$ left the rumen was considered to be ruminally undegradable. Post-ruminal $InsP_6$ degradation was found to occur mainly in the upper small intestine (between the omasum + abomasum and jejunum) in the present study. It was assumed that a part of rumen undegradable $InsP_6$, which was probably bound in protein-phytate complexes (see Chapter 5.2.2), was released through the enzymatic degradation of rumen undegradable protein (RUP) in the abomasum and upper part of the small intestine. As mucosal phytase and phosphatase activity has been determined in the small intestine of calf (Bitar and Reinhold, 1972) and in beef cattle (Jay and Ray, 1972), and 6 bacterial genera positively related to P digestibility (*Desulfovibrio*, *Fibrobacter*, *Prevotella*, *Rikenellaceae_RC9_gut_group*, *Ruminococcus_2* and *Victivallis*) have been found in

the jejunum of goats (Wang et al., 2020), these might have contributed to the degradation of $InsP_6$ released in the small intestine.

However, it seems that post-ruminal degradation of $InsP_6$ is limited, as neither in the present study nor in the aforementioned studies, rumen undegraded $InsP_6$ was completely degraded before reaching the rectum. In Manuscript 1, $InsP_6$ disappearance up to the rectum was 82 and 93% for wethers fed a diet containing RSM and SBM, respectively. Brask-Pedersen et al. (2013) observed reduced flow of $InsP_6$-P between the duodenum and ileum (from 3.8 to 2.6 g/d), but there was no further reduction of $InsP_6$-P posterior to the ileum (2.6 g/d in the ileum and 2.7 g/d in the faeces). In their study, 10% of dietary $InsP_6$-P was quantified in the faeces of cows. In the study by Park et al. (2002), 7% of dietary $InsP_6$ reached the rectum of sheep, despite the higher extent of post-ruminal $InsP_6$ degradation in the upper large intestine. These results support the assertion made by Ray et al. (2012) that microbial phytase activity in the large intestine is less efficient compared to the rumen, which might be explained by the faster passage and more homogenous composition of digesta, and a lower fill capacity of the large intestine (Mambrini and Peyraud, 1997). In the study by Ray et al. (2012), 14−17% of $InsP_6$ infused into the ileum was degraded in the large intestine, without significant difference between the infusion rates of $InsP_6$. Ray et al. (2013) suggested a constant value of 16% for large intestinal $InsP_6$ disappearance (based on flow of $InsP_6$ into the large intestine), which was not affected by $InsP_6$ amount in the diet. These results are in good accordance with the 19% disappearance determined for $InsP_6$ entering the large intestine by Park et al. (2002). According to the present results and results from previous studies, it can be concluded that a part of potentially rumen degradable $InsP_6$ can be further degraded in the large intestine, as certain rumen microbial population exhibiting phytase activity, such as *Prevotella* spp., is also present in the large intestine (Mao et al., 2015; Purushe et al., 2010). Nevertheless, rumen undegradable $InsP_6$ remains mostly undegraded when passing through the post-ruminal digestive sections. Furthermore, P absorption posterior to the ileum seems to be of low relevance (GfE, 2023), which implies that the P released from $InsP_6$ degradation in the large intestine would not be beneficial for animals.

Based on the aforementioned aspects, factors inhibiting ruminal $InsP_6$ degradation may be applied to post-ruminal $InsP_6$ degradation. For oilseed meals, the interaction between $InsP_6$ and CP and the correlation between $InsP_6$ and CP degradation (see Chapter 5.2.2)

are likely to cause the conflicted interest of oilseed processing which is commonly adopted to increase the flow of dietary AA through RUP into the small intestine for a higher milk protein yield. In contrast to RUP, which demonstrates high intestinal digestibility, as shown in Manuscript 2 for SBM (averaging 93%; ranging from 87 to 96%) and in the study by Steingass et al. (2013) for RSM (averaging 80%; ranging from 75 to 84%), the rumen undegradable $InsP_6$ would to be largely excreted in the faeces.

5.4 DIFFERENCE AMONG INDIVIDUALS

As addressed in Chapter 4.1, one objective of the present study was to evaluate the individual difference between animals fed diets containing different oilseed meals. Previous studies from the Department of Animal Nutrition at University of Hohenheim (data not published) observed a variation in total tract $InsP_6$ disappearance between 70 and 84% in 6 wethers and between 81 and 92% in 22 cows fed RSM-containing diet. For SBM-containing diet, total tract $InsP_6$ disappearance varied between 93 and 96% in 6 wethers and between 90 and 96% in 23 cows. In Manuscript 1, the data were calculated from each wether and a greater individual variation in ruminal and total tract $InsP_6$ disappearance, disappeared $InsP_6$ amount, and blood MI was observed for wethers fed a diet containing RSM compared to those fed a diet containing SBM (Table 4). Up to now, published data have yet to report this phenomenon upon feeding RSM.

Although slight variation of the data caused by scientific methods or random animal factors could not be ruled out (see Chapter 5.1), a possible explanation for these observations could be the microbial population in the digestive tract of individual animals. Microorganisms are essential for $InsP_6$ degradation in ruminants (Lan et al., 2002a; Yanke et al., 1998), yet bacterial composition and abundance vary widely even when animals are fed the same diet and kept in the same environment (Jami and Mizrahi, 2012). Recently, Wang et al. (2020) observed a varied P digestibility ranging from 68 to 90% in 28 goats despite the similar feeding, genetic background, and age. As localization, binding form and interaction with protein of $InsP_6$ in RSM seems to hinder $InsP_6$ degradation to a greater extent, it is possible that the individual microbial environment of animals plays a more important role during $InsP_6$ degradation than when sources of easily degradable $InsP_6$, such as SBM, are fed.

Table 4. Phytate ($InsP_6$) disappearance, disappeared $InsP_6$ amount, and blood *myo*-inositol concentration in wethers fed a diet containing either rapeseed meal (RSM) or soybean meal (SBM).[*]

	Diet containing RSM				Diet containing SBM			
	Mean	Min	Max	SD	Mean	Min	Max	SD
$InsP_6$ disappearance (%)								
Ruminal[#]	76	68	89	9.32	89	88	89	0.42
Total tract[§]	82	76	94	7.97	93	88	95	2.91
Disappeared $InsP_6$ amount (g/d)								
Ruminal	4.50	4.01	5.30	0.55	3.39	3.37	3.40	0.02
Total tract	4.88	4.48	5.56	0.47	3.54	3.38	3.64	0.11
Blood *myo*-inositol (μg/ml)	4.56	4.15	5.30	0.51	3.78	3.29	4.33	0.44

[*]Data are presented as mean, minimal, and maximal values with standard deviation (SD) (n = 4 wethers).

[#]Ruminal data were calculated with respective $InsP_6$ disappearance or disappeared $InsP_6$ amount in fractions of omasum and abomasum on a dry matter basis.

[§]Total tract data were calculated with $InsP_6$ disappearance or disappeared $InsP_6$ amount up to the rectum.

5.5 CONCLUSIONS

Ruminal $InsP_6$ degradation differed widely when animals were fed a diet containing RSM or a diet containing SBM. The easily degradable $InsP_6$ in SBM compared to RSM rendered the extent of ruminal $InsP_6$ disappearance for a diet containing SBM higher than a diet containing RSM. In contradiction to the hypothesis that post-ruminal $InsP_6$ degradation is of higher relevance in case of an incomplete ruminal $InsP_6$ degradation, the observed extent of post-ruminal $InsP_6$ degradation was very low and did not differ between diets. Because of the low rumen passage rate, it was suggested that the degradable fraction of $InsP_6$ from feed was entirely degraded in the rumen, and $InsP_6$ which left the rumen remained mainly undegradable until being excreted.

In situ determined ruminal $InsP_6$ degradation varied considerably among SBM from different origins. Similar to RSM of the previous study, there is also a close relationship between $InsP_6$ degradation and CP degradation, which can be used for predicting $InsP_6$ degradation. However, feedstuff-specific equations may be necessary and validation with a larger data set is required before predictions can be applied in practice.

The effect of $InsP_6$ amount on *in vitro* $InsP_6$ degradation differed between RSM and SBM, which may be ascribed to the internal structure and nutrient composition of the feed that influence degradation kinetics of $InsP_6$. However, other experimental factors and way of result expression seem to affect the interpretation regarding effect of $InsP_6$ amount. To reflect the real degradation kinetics, it is recommended to compare $InsP_6$ degradation based on the amount of $InsP_6$ in the feed and express the data as amount of $InsP_6$ rather than relative values.

Overall, results of the present study showed a high similarity among ruminal $InsP_6$ degradation determined by using *in vivo*, *in situ*, and *in vitro* methods. This implies that evaluation of ruminal $InsP_6$ degradation based on *in situ* or *in vitro* study may be applied to *in vivo* conditions. However, standard procedures should be established to minimise variability between studies and to increase reproducibility of the data. Further research about phytase-producing microbial population and their phytase activity would be helpful to understand the response of $InsP_6$ degradation between diets and other influencing factors.

6 SUMMARY

Oilseed meals are widely used protein feeds in ruminant nutrition. However, aside from the high crude protein (CP) content, oilseed meals also contain high amounts of phosphorus (P), which is predominantly present in organic form as different salts of *myo*-inositol 1,2,3,4,5,6 hexakis dihydrogen phosphate ($InsP_6$). To become available for intestinal absorption and further utilisation by animals, P must be cleaved from the $InsP_6$ molecule by a specific group of phosphatases, which is known as phytase. Over the decades, ruminants were considered to be capable of utilising nearly all P bound in $InsP_6$ because of the substantial phytase activity exhibited by rumen microbiota. Nevertheless, recent studies have reported variable extents of ruminal $InsP_6$ degradation which seems to be influenced by different factors. In case of an incomplete ruminal $InsP_6$ degradation, post-ruminal $InsP_6$ degradation may be of higher relevance. However, post-ruminal $InsP_6$ degradation has been rarely studied to date.

The aim of this thesis was to systematically investigate $InsP_6$ degradation of rapeseed meal (RSM) and soybean meal (SBM) in ruminants, including the possible influencing factors and their combinations. Different study methods (*in vivo*, *in situ*, and *in vitro*) were applied to evaluate the effects of RSM and SBM.

The first study (Manuscript 1) was conducted to investigate ruminal and post-ruminal $InsP_6$ degradation in wethers fed a diet containing RSM or SBM, and to link the ruminal disappearance determined in slaughtered wethers with *in situ* calculated rumen effective degradation of $InsP_6$ ($InsP_6ED$) from cows. Firstly, RSM and SBM was incubated according to a standard *in situ* procedure in three lactating Jersey cows for 2, 4, 6, 8, 16, 24, 48, and 72 h to obtain $InsP_6ED$ for the oilseed meals at rumen passage rates of 0.02 ($InsP_6ED_2$) and 0.05 h^{-1} ($InsP_6ED_5$). Secondly, eight wethers were randomly assigned to two treatment groups that were fed a diet containing equal amount of RSM (Diet RSM) or SBM (Diet SBM) for 8 weeks of adaptation. Then, digesta from the reticulo-rumen, omasum, abomasum, jejunum, colon, and rectum were sampled. In consistence with *in situ* calculated $InsP_6ED_2$ (83 and 93% for RSM and SBM, respectively), ruminal $InsP_6$ disappearance was lower in wethers fed Diet RSM (76%) compared to those fed Diet SBM (89%). Post-ruminal $InsP_6$ disappearance did not differ between dietary treatments (6% for Diet RSM vs. 4% for Diet SBM). A higher amount of ruminally degraded $InsP_6$ was observed upon feeding RSM (4.5 g/d for Diet

RSM and 3.4 g/d for Diet SBM). Due to the low rumen passage rate in this study, it was suggested that P from $InsP_6$ being available to ruminants is almost entirely from $InsP_6$ degradation in the rumen.

As $InsP_6$ is located in a protein-rich structure in seeds and $InsP_6$ degradation has been recently reported to vary in a pattern similar to CP degradation for RSM, the second study (Manuscript 2) was carried out to investigate the variation of *in situ* ruminal $InsP_6$ degradation of SBM and its relation to CP degradation. In this study, nine commercial solvent-extracted SBM from Europe and South America were incubated in three rumen-fistulated lactating Jersey cows with the same procedure performed in the first study. Rumen effective degradation of CP and $InsP_6$ were calculated for a rumen passage rate of 0.06 h^{-1} ($CPED_6$ and $InsP_6ED_6$). Chemical protein fractions of SBM variants were determined according to Cornell Net Carbohydrate and Protein System (CNCPS). The SBM variants exhibited a considerable variation in CP and $InsP_6$ degradation. Significant correlations were found between $InsP_6ED_6$ and $CPED_6$ and between $InsP_6ED_6$ and all CNCPS protein fractions, which confirmed the close relationship between CP and $InsP_6$ degradation for SBM. The results suggested that using a general value of $InsP_6$ degradation for diet formulation may not be precise enough, and $InsP_6ED$ may be predicted based on CPED or CNCPS protein fractions by using linear regression equations.

The third study (Chapter 4.3) aimed to achieve a better understanding of how *in vitro* $InsP_6$ degradation of RSM and SBM is influenced by different amounts of $InsP_6$ in feed. The same batches of RSM and SBM as used in Manuscript 1 were incubated in a modified rumen simulation technique (RUSITEC) system with different amounts for 3, 6, 12, 24, and 48 h. Degradation of $InsP_6$ from bag residues was calculated and expressed as amount and in percentage using the same equation as applied for *in situ* calculations. *In vitro* degradation of $InsP_6$ in response to $InsP_6$ amount differed between RSM and SBM, which may be attributed to the different internal structure and nutrient composition of the oilseed meals. Only when expressing in amounts, the calculated $InsP_6ED$ was observed to increase linearly with increasing $InsP_6$ amount in feed. Accordingly, it was recommended to compare $InsP_6$ degradation based on $InsP_6$ amount in the feed and to express degradation as amount instead of using relative value which might not reflect the real degradation kinetics.

In conclusion, the results of this thesis showed that the extent of ruminal $InsP_6$ degradation differs when the diet contains either RSM or SBM, while post-ruminal $InsP_6$ degradation is negligibly low given a long rumen retention time. By using linear regression equations, ruminal $InsP_6$ degradation may be predicted from CP degradation due to the close relationship therebetween. Effects of $InsP_6$ amount on $InsP_6$ degradation is dependent on $InsP_6$ source. Based on the high similarity among ruminal $InsP_6$ degradation determined by different methods in this thesis, ruminal $InsP_6$ degradation of oilseed meals measured by *in situ* or *in vitro* study may be applicable for *in vivo* conditions.

7 ZUSAMMENFASSUNG

Ölschrote sind sehr relevante Proteinfuttermittel in der Wiederkäuerernährung. Neben dem hohen Gehalt an Rohprotein (CP) enthalten Ölschrote jedoch auch einen hohen Anteil an Phosphor (P), der vorwiegend in organischer Form als verschiedene Salze der Phytinsäure vorliegt (*Myo*-Inositol 1,2,3,4,5,6 Hexakisdihydrogenphosphat, $InsP_6$). Um für die Absorption im Darm und die weitere Verwertung durch Tiere verfügbar zu werden, muss P mithilfe der Phytasen vom $InsP_6$-Molekül abgespalten werden. Über die Jahrzehnte hinweg wurde angenommen, dass Wiederkäuer aufgrund der erheblichen Phytaseaktivität des Pansenmikrobioms in der Lage sind, nahezu den gesamten an $InsP_6$ gebundenen P freizusetzen. Dennoch haben aktuelle Studien unterschiedliche Ausmaße des ruminalen $InsP_6$-Abbaus gezeigt, die offenbar von verschiedenen Faktoren beeinflusst werden. Im Falle eines unvollständigen ruminalen $InsP_6$-Abbaus könnte der postruminale $InsP_6$-Abbau von großer Bedeutung sein. Allerdings wurde der postruminale $InsP_6$-Abbau bisher nur selten erforscht.

Das übergeordnete Ziel dieser Arbeit war es, den $InsP_6$-Abbau von Rapsextraktionsschrot (RSM) und Sojaextraktionsschrot (SBM) bei Wiederkäuern systematisch zu untersuchen, einschließlich der möglichen Einflussfaktoren und ihrer Kombinationen. Verschiedene Untersuchungsmethoden (*in vivo*, *in sit*u und *in vitro*) wurden angewendet, um die Effekte zu evaluieren.

Die erste Studie (Manuskript 1) wurde durchgeführt, um den ruminalen und postruminalen $InsP_6$-Abbau bei Hammeln zu untersuchen, die mit einer Ration aus RSM oder SBM gefüttert wurden. Darüber hinaus wurde versucht, das ruminale Verschwinden von $InsP_6$ bei Hammeln mit dem *in situ* berechneten ruminalen effektiven Abbau von $InsP_6$ ($InsP_6ED$), bestimmt bei Kühen, zu verknüpfen. Zunächst wurden RSM und SBM gemäß eines standardmäßigen *in situ* Verfahrens bei drei laktierenden Jersey-Kühen für 2, 4, 6, 8, 16, 24, 48 und 72 Stunden inkubiert, um Werte für $InsP_6ED$ für beide Ölschrote bei einer Passage durch den Pansen von 0,02 ($InsP_6ED_2$) und 0,05 h^{-1} ($InsP_6ED_5$) zu erhalten. Zweitens wurden acht Hammel zufällig zwei Behandlungsgruppen zugeordnet, die über einen Adaptionszeitraum von 8 Wochen mit einer Ration gefüttert wurden, die eine gleiche Menge an RSM (Diet RSM) oder SBM (Diet SBM) enthielt. Anschließend wurde die Digesta aus dem Pansen, Omasum, Abomasum, Jejunum, Kolon und Rektum entnommen. In Übereinstimmung mit den *in*

*sit*u berechneten $InsP_6ED_2$-Werten (83% für RSM und 93% für SBM) war das ruminale Verschwinden von $InsP_6$ bei den mit der Diet RSM gefütterten Hammeln (76%) niedriger im Vergleich zu denen, die die Diet SBM erhielten (89%). Das postruminale Verschwinden von $InsP_6$ unterschied sich hingegen nicht zwischen den beiden Behandlungen (6% für Diet RSM vs. 4% für Diet SBM). Bei der Fütterung von RSM wurde eine höhere Menge an ruminal abgebautem $InsP_6$ bei Hammeln beobachtet (4,5 g/Tag für Diet RSM und 3,4 g/Tag für Diet SBM). Aufgrund der niedrigen Passagerate im Pansen wurde angenommen, dass P aus $InsP_6$ im Futter fast ausschließlich durch den $InsP_6$-Abbau im Pansen für Wiederkäuer verfügbar wird.

Da sich $InsP_6$ in einer proteinreichen Struktur in den Samen befindet und bei RSM kürzlich über ein ähnliches Muster der Variation zwischen dem $InsP_6$-Abbau und dem CP-Abbau berichtet wurde, wurde die zweite Studie (Manuskript 2) durchgeführt, um die Variation des *in situ* ruminalen $InsP_6$-Abbaus von SBM und deren Beziehung zum CP-Abbau zu untersuchen. In dieser Studie wurden neun kommerziell lösungsmittelextrahierte SBM aus Europa und Südamerika in drei pansenfistulierten laktierenden Jersey-Kühen mit dem gleichen Verfahren wie in der ersten Studie inkubiert. Der ruminale effektive Abbau von CP und $InsP_6$ wurden für eine Pansenpassagerate von 0,06 h^{-1} ($CPED_6$ und $InsP_6ED_6$) berechnet. Die chemischen Proteinfraktionen der SBM-Varianten wurden nach dem Cornell Net Carbohydrate and Protein System (CNCPS) bestimmt. Die SBM-Varianten wiesen eine erhebliche Variation im CP- und $InsP_6$-Abbau auf. Es wurden signifikante Korrelationen zwischen $InsP_6ED_6$ und $CPED_6$ sowie zwischen $InsP_6ED_6$ und allen CNCPS-Proteinfraktionen ermittelt. Die Ergebnisse deuten darauf hin, dass ein einzelner genereller Wert des $InsP_6$-Abbaus für die Rationsgestaltung möglicherweise nicht präzise genug ist, und dass $InsP_6ED$ anhand von CPED oder CNCPS-Proteinfraktionen mithilfe linearer Regressionsgleichungen geschätzt werden kann.

In der dritten Studie (Manuskript 3) wurde untersucht, wie der *in vitro* $InsP_6$-Abbau von RSM und SBM von verschiedenen Mengen an $InsP_6$ im Futter beeinflusst wird. Dieselben Chargen von RSM und SBM, die in Manuskript 1 verwendet wurden, wurden in einem modifizierten Pansensimulationssystem (RUSITEC) mit verschiedenen Mengen für 3, 6, 12, 24 und 48 Stunden inkubiert. Der $InsP_6$-Abbau aus den Beutelrückständen wurde sowohl in Mengen als auch in Prozent der inkubierten Menge ausgedrückt. Der *in vitro* $InsP_6$-Abbau unter Einfluss von der $InsP_6$-Menge

unterschied sich signifikant zwischen RSM und SBM, was auf die unterschiedliche innere Struktur und Nährstoffzusammensetzung des Futters zurückgeführt werden kann. Nur bei der Angabe der Menge wurde beobachtet, dass die berechnete $InsP_6$ED linear mit zunehmender $InsP_6$-Menge im Futter anstieg. Dementsprechend wurde empfohlen, den $InsP_6$-Abbau anhand der $InsP_6$-Menge im Futter zu vergleichen und den Abbau als Menge anzugeben, anstatt relative Werte zu verwenden, die möglicherweise nicht die Abbaukinetik widerspiegeln.

Zusammenfassend zeigten die Ergebnisse dieser Arbeit, dass der ruminale $InsP_6$-Abbau sich zwischen einer RSM- und SBM-enthaltenden Ration unterscheidet, während der postruminale $InsP_6$-Abbau bei einer langen Verweilzeit im Pansen vernachlässigbar gering und nicht unterschiedlich ist. Mithilfe linearer Regressionsgleichungen kann der ruminale $InsP_6$-Abbau aufgrund der engen Beziehung aus dem CP-Abbau geschätzt werden. Die Effekte der $InsP_6$-Menge auf den $InsP_6$-Abbau sind abhängig von der $InsP_6$-Quelle. Basierend auf der hohen Übereinstimmung zwischen den Ergebnissen des ruminalen $InsP_6$-Abbaus, die mit verschiedenen Methoden ermittelt wurden, kann der durch *in situ* oder *in vitro* bestimmte ruminale $InsP_6$-Abbau von Ölschroten auf *in vivo*-Bedingungen anwendbar sein.

8 REFERENCES (USED IN CHAPTERS 1, 2, 3, AND 5)

Ahvenjärvi, S., Vanhatalo, A., Hristov, A.N., Huhtanen, P., 2004. Passage kinetics of internal and external markers in lactating dairy cows. J. Anim. Feed Sci. 13, 19–22.

Ahvenjärvi, S., Vanhatalo, A., Huhtanen, P., Varvikko, T., 2000. Determination of reticulo-rumen and whole-stomach digestion in lactating cows by omasal canal or duodenal sampling. Br. J. Nutr. 83, 67–77.

Ahvenjärvi, S., Vanhatalo, A., Shingfield, K.J., Huhtanen, P., 2003. Determination of digesta flow entering the omasal canal of dairy cows using different marker systems. Br. J. Nutr. 90, 41–52.

Amat, T., Assifaoui, A., Schmitt, C., Saurel, R., 2022. Importance of binary and ternary complex formation on the functional and nutritional properties of legume proteins in presence of phytic acid and calcium. Crit. Rev. Food Sci. Nutr. 63, 1–23.

Anderson, R.L., Wolf, W.J., 1995. Compositional changes in trypsin inhibitors, phytic acid, saponins and isoflavones related to soybean processing. J. Nutr. 125, 581–588.

Bailey, C.B., 1961. Saliva secretion and its relation to feeding in cattle: the relationship between the concentrations of sodium, potassium, chloride and inorganic phosphate in mixed saliva and rumen fluid. Br. J. Nutr. 15, 489–498.

Bayat, A.R., Rinne, M., Khalili, H., Valizadeh, R., Huhtanen, P., 2007. Estimation of digesta kinetics of different particle size fractions using rumen evacuation technique in dairy cows fed red clover-grass silage. J. Anim. Feed Sci. 16, 538–554.

Bayat, A.R., Rinne, M., Kuoppala, K., Ahvenjärvi, S., Huhtanen, P., 2011. Ruminal large and small particle kinetics in dairy cows fed primary growth and regrowth grass silages harvested at two stages of growth. Anim. Feed Sci. Technol. 165, 51–60.

Becker, R.B., Neal, W.M., Shealy, A.L., York, G., 1933. Stiffs or sweeny (phosphorus deficiency) in cattle. Univ. Florida Agric. Exp. Stat., Bulletin No. 264, 27.

Bitar, K., Reinhold, J.G., 1972. Phytase and alkaline phosphatase activities in intestinal mucosae of rat, chicken, calf, and man. Biochim. Biophys. Acta. 268, 442–452.

Black, W.H., Tash, L.H., Jones, J.M., Kleberg Jr, R.J., 1943. Effects of phosphorus supplements on cattle grazing on range deficient in this mineral. USDA Technical, Bulletin No. 856.

Boguhn, J., Kluth, H., Rodehutscord, M., 2006. Effect of total mixed ration composition on fermentation and efficiency of ruminal microbial crude protein synthesis in vitro. J. Dairy Sci. 89, 1580–1591.

Boguhn, J., Zuber, T., Rodehutscord, M., 2013. Effect of donor animals and their diet on in vitro nutrient degradation and microbial protein synthesis using grass and corn silages. J. Anim. Physiol. Anim. Nutr. 97, 547–557.

Brask-Pedersen, D.N., Glitsø, L.V., Skov, L.K., Lund, P., Sehested, J., 2013. Effect of exogenous phytase on degradation of inositol phosphate in dairy cows. J. Dairy Sci. 96, 1691–1700.

Brask-Pedersen, D.N., Glitsø, L.V., Skov, L.K., Lund, P., Sehested, J., 2011. Effect of exogenous phytase on feed inositol phosphate hydrolysis in an in vitro rumen fluid buffer system. J. Dairy Sci. 94, 951–959.

Bravo, D., Sauvant, D., Bogaert, C., Meschy, F., 2003. II. Quantitative aspects of phosphorus absorption in ruminants. Reprod. Nutr. Dev. 43, 271–284.

Breves, G., Schröder, B., 1991. Comparative aspects of gastrointestinal phosphorus metabolism. Nutr. Res. Rev. 4, 125–140.

Brooks, J.R., Morr, C.V., 1982. Phytate removal from soy protein isolates using ion exchange processing treatments. J. Food Sci. 47, 1280–1282.

Bruce, J., Goodall, E.D., Kay, R.N., Phillipson, A.T., Vowles, L.E., 1966. The flow of organic and inorganic materials through the alimentary tract of the sheep. Proc. R. Soc. B. Biol. Sci. 166, 46–62.

Camacho, L.F., da Silva, T.E., Rodrigues, J.P.P., Franco, M. de O., Detmann, E., 2022. A standard procedure for in vitro digestion using rumen fermenters: a collaborative study. Animals 12, 2842.

Care, A.D., 1994. The absorption of phosphate from the digestive tract of ruminant animals. Br. Vet. J. 150, 197–205.

Carrizo, S.L., de Oca, C.E.M., Laiño, J.E., Suarez, N.E., Vignolo, G., LeBlanc, J.G., Rollán, G., 2016. Ancestral Andean grain quinoa as source of lactic acid bacteria capable to degrade phytate and produce B-group vitamins. Food Res. Int. 89, 488–494.

Cherney, D.J.R., Patterson, J.A., Lemenager, R.P., 1990. Influence of in situ bag rinsing technique on determination of dry matter disappearance. J. Dairy Sci. 73, 391–397.

Cheryan, M., 1980. Phytic acid interactions in food systems. Crit. Rev. Food Sci. Nutr. 13, 297–335.

Cheryan, M., Anderson, F.W., Grynspan, F., 1983. Magnesium-phytate complexes: effects of pH and molar ratio on solubility characteristics. Cereal Chem. 60, 235–237.

Chung, S.Y., Han, S.H., Lee, S.W., Rhee, C., 2012. Effect of Maillard reaction products prepared from glucose–glycine model systems on starch digestibility. Starch - Stärke 64, 657–664.

Clark, W.D., Wohlt, J.E., Gilbreath, R.L., Zajac, P.K., 1986. Phytate phosphorus intake and disappearance in the gastrointestinal tract of high producing dairy cows. J. Dairy Sci. 69, 3151–3155.

Correll, D.L., 1998. The role of phosphorus in the eutrophication of receiving waters: a review. J. Environ. Qual. 27, 261–266.

Damiran, D., DelCurto, T., Bohnert, D.W., Findholt, S.L., 2008. Comparison of techniques and grinding size to estimate digestibility of forage based ruminant diets. Anim. Feed Sci. Technol. 141, 15–35. ht

Dennison, C., Marais, J.P., 1980. The influence of ruminant salivary buffer salts upon the in vitro microbial digestion of forages. S. Afr. J. sci 12, 6.

Diao, X., Dang, S., Liu, S., Jing, L., Wang, Y., Zhang, W., 2020. Determination of the appropriate ratio of sample size to nylon bag area for in situ nylon bag technique evaluation of rumen digestibility of feedstuffs in sheep. Livest. Sci. 241, 104254.

Durand, M., Komisarczuk, S., 1988. Influence of major minerals on rumen microbiota. J. Nutr. 118, 249–260.

Eeckhout, W., de Paepe, M., 1994. Total phosphorus, phytate-phosphorus and phytase activity in plant feedstuffs. Anim. Feed Sci. Technol. 47, 19–29.

Escobar, N., Tizado, E.J., zu Ermgassen, E.K.H.J., Löfgren, P., Börner, J., Godar, J., 2020. Spatially-explicit footprints of agricultural commodities: mapping carbon emissions embodied in Brazil's soy exports. Glob. Environ. Change 62, 102067.

Faichney, G.J., 1980. The use of markers to measure digesta flow from the stomach of sheep fed once daily. J. Agric. Sci. 94, 313–318.

Faichney, G.J., Poncet, C., Boston, R.C., Bernard, L., Pochet, S., Beaufort, M.-T., Delval, E., Fabre, M., Pichon, P., Flechet, J., 1989. Passage of internal and external markers of particulate matter through the rumen of sheep. Reprod. Nutr. Dev. 29, 325–337.

FAO, 2021. OECD-FAO Agricultural outlook 2021-2030. Paris.

Fatehi, F., Krizsan, S.J., Gidlund, H., Huhtanen, P., 2015. A comparison of ruminal or reticular digesta sampling as an alternative to sampling from the omasum of lactating dairy cows. J. Dairy Sci. 98, 3274–3283.

Fontaine, T.D., Pons, W.A., Irving, G.W., 1946. Protein-phytic acid relationship in peanuts and cottonseed. J. Biol. Chem. 164, 487–507.

Foskolos, A., Ferret, A., Siurana, A., Castillejos, L., Calsamiglia, S., 2020. Effects of Capsicum and Propyl-propane thiosulfonate on rumen fermentation, digestion, and milk production and composition in dairy cows. Animals 10, 859.

GfE [Gesellschaft für Ernährungsphysiologie], 2023. Empfehlungen zur Energie- und Nährstoffversorgung von Milchkühen. Frankfurt am Main, Germany: DLG-Verlag.

GfE [Gesellschaft für Ernährungsphysiologie], 2022. Recommended protocol for the determination of nutrient disappearance in situ for estimation of ruminal degradation. Proc. Soc. Nutr. Physiol. 31, 177–189.

GfE [Gesellschaft für Ernährungsphysiologie], 2001. Empfehlungen zur Energie- und Nährstoffversorgung der Milchkühe und Aufzuchtrinder. Frankfurt am Main, Germany: DLG-Verlag.

Gillberg, L., Törnell, B., 1976. Preparation of rapeseed protein isolates. Dissolution and precipitation behavior of rapeseed proteins. J. Food Sci. 41, 1063–1069.

Gillespie, J., Rogers, S.W., Deery, M., Dupree, P., Rogers, J.C., 2005. A unique family of proteins associated with internalized membranes in protein storage vacuoles of the Brassicaceae. Plant J. 41, 429–441.

Godoy, S., Meschy, F., 2001. Utilisation of phytate phosphorus by rumen bacteria in a semi-continuous culture system (Rusitec) in lactating goats fed on different forage to concentrate ratios. Reprod. Nutr. Dev. 41, 259–265.

Greiner, R., Konietzny, U., 2005. Phytase for food application. Food Technol. Biotechnol. 44, 125–140.

Grynspan, F., Cheryan, M., 1983. Calcium phytate: effect of ph and molar ratio on in vitro solubility. J. Am. Oil Chem. Soc. 60, 1761–1764.

Haese, E., Krieg, J., Grubješić, G., Feyder, A., Rodehutscord, M., 2020. Determination of in situ ruminal degradation of phytate phosphorus from single and compound feeds in dairy cows using chemical analysis and near-infrared spectroscopy. Animal 14, 1461–1471.

Haese, E., Lengowski, M., Gräter, E., Föll, A., Möhring, J., Steingass, H., Schollenberger, M., Rodehutscord, M., 2017a. Ruminal phytate degradation of maize grain and rapeseed meal in vitro and as affected by phytate content in donor animal diets and inorganic phosphorus in the buffer. J. Anim. Physiol. Anim. Nutr. 101, 868–880.

Haese, E., Möhring, J., Steingass, H., Schollenberger, M., Rodehutscord, M., 2017b. Effect of dietary mineral phosphorus and phytate on in situ ruminal phytate disappearance from different concentrates in dairy cows. J. Dairy Sci. 100, 3672–3684.

Haese, E., Müller, K., Steingass, H., Schollenberger, M., Rodehutscord, M., 2014. Effects of mineral and rapeseed phosphorus supplementation on phytate degradation in dairy cows. Arch. Anim. Nutr. 68, 478–491.

Haese, E., Titze, N., Rodehutscord, M., 2022. In situ ruminal disappearance of crude protein and phytate from differently processed rapeseed meals in dairy cows. J. Sci. Food Agric. 102, 2805–2812.

Haros, M., Bielecka, M., Sanz, Y., 2005. Phytase activity as a novel metabolic feature in Bifidobacterium. FEMS Microbiol. Lett. 247, 231–239.

Hídvégi, M., Lásztity, R., 2002. Phytic acid content of cereals and legumes and interaction with proteins. Period. Polytech. Chem. Eng. 46, 59–64.

Hu, H.L., Wise, A., Henderson, C., 1996. Hydrolysis of phytate and inositol tri-, tetra-, and penta- phosphates by the intestinal mucosa of the pig. Nutr. Res. 16, 781–787.

Huang, H., Zhang, R., Fu, D., Luo, J., Li, Z., Luo, H., Shi, P., Yang, P., Diao, Q., Yao, B., 2011. Diversity, abundance and characterization of ruminal cysteine phytases suggest their important role in phytate degradation. Environ. Microbiol. 13, 747–757.

Huber, K., 2016. Cellular myo-inositol metabolism. Pages 53-60. In: Walk CL, Kühn I, Stein HH, Kidd MT, Rodehutscord M, editors. Phytate destruction - consequences for precision animal nutrition. Wageningen Academic Publishers, Wageningen, The Netherlands.

Huhtanen, P., Asikainen, U., Arkkila, M., Jaakkola, S., 2007. Cell wall digestion and passage kinetics estimated by marker and in situ methods or by rumen evacuations in cattle fed hay 2 or 18 times daily. Anim. Feed Sci. Technol. 133, 206–227.

Isayenkov, S., 2014. Plant vacuoles: physiological roles and mechanisms of vacuolar sorting and vesicular trafficking. Cytol. Genet. 48, 127–137.

Ishiguro, T., Ono, T., Wada, T., Tsukamoto, C., Kono, Y., 2006. Changes in soybean phytate content as a result of field growing conditions and influence on tofu texture. Biosci. Biotechnol. Biochem. 70, 874–80.

Jami, E., Mizrahi, I., 2012. Composition and similarity of bovine rumen microbiota across individual animals. PLoS One 7, e33306.

Jay, A.E., Ray, M.L., 1972. Relationship of selected cations to mucosal amino acid absorption and phosphatase activity by isolated bovine jejunal segments. J. Anim. Sci. 34, 805–808.

Jiang, L., Phillips, T.E., Hamm, C.A., Drozdowicz, Y.M., Rea, P.A., Maeshima, M., Rogers, S.W., Rogers, J.C., 2001. The protein storage vacuole. J. Cell Biol. 155, 991–1002.

Karlsson, J.O., Parodi, A., van Zanten, H.H.E., Hansson, P.-A., Röös, E., 2021. Halting European Union soybean feed imports favours ruminants over pigs and poultry. Nat. Food 2, 38–46.

Kies, A.K., De Jonge, L.H., Kemme, P.A., Jongbloed, A.W., 2006. Interaction between protein, phytate, and microbial phytase. In vitro studies. J. Agric. Food Chem. 54, 1753–1758.

Kincaid, R.L., Garikipati, D.K., Nennich, T.D., Harrison, J.H., 2005. Effect of grain source and exogenous phytase on phosphorus digestibility in dairy cows. J. Dairy Sci. 88, 2893–2902.

Kolláthová, R., Varga, B., Ivanišová, E., Biro, D., Rolinec, M., Juracek, M., Šimko, M., Gálik, B., 2019. Mineral profile analysis of oilseeds and their by-products as feeding sources for animal nutrition. Slovak J. Anim. Sci. 52, 9–15.

Komisarczuk, S., Merry, R.J., McAllan, A.B., 1987. Effect of different levels of phosphorus on rumen microbial fermentation and synthesis determined using a continuous culture technique. Br. J. Nutr. 57, 279–290.

Konishi, C., Matsui, T., Park, W., Yano, H., Yano, F., 1999. Heat treatment of soybean meal and rapeseed meal suppresses rumen degradation of phytate phosphorus in sheep. Anim. Feed Sci. Technol. 80, 115–122.

Krizsan, S.J., Ahvenjärvi, S., Huhtanen, P., 2010. A meta-analysis of passage rate estimated by rumen evacuation with cattle and evaluation of passage rate prediction models. J. Dairy Sci. 93, 5890–5901.

Künzel, S., Yergaliyev, T., Wild, K.J., Philippi, H., Petursdottir, A.H., Gunnlaugsdottir, H., Reynolds, C.K., Humphries, D.J., Camarinha-Silva, A., Rodehutscord, M., 2022. Methane reduction potential of brown seaweeds and their influence on nutrient degradation and microbiota composition in a rumen simulation technique. Front. Microbiol. 13, 889618.

Lamid, M., Al-Arif, A., Asmarani, O., Warsito, S.H., 2018. Characterization of phytase enzymes as feed additive for poultry and feed. IOP Conf. Ser.: Earth Environ. Sci. 137, 012009.

Lan, G.Q., Abdullah, N., Jalaludin, S., Ho, Y.W., 2002a. Optimization of carbon and nitrogen sources for phytase production by Mitsuokella jalaludinii, a new rumen bacterial species. Lett. Appl. Microbiol. 35, 157–161.

Lan, G.Q., Ho, Y.W., Abdullah, N., 2002b. Mitsuokella jalaludinii sp. nov., from the rumens of cattle in Malaysia. Int. J. Syst. Evol. Microbiol. 52, 713–718.

Lan, X., Liu, P., Xia, S., Jia, C., Mukunzi, D., Zhang, X., Xia, W., Tian, H., Xiao, Z., 2010. Temperature effect on the non-volatile compounds of Maillard reaction products derived from xylose–soybean peptide system: Further insights into thermal degradation and cross-linking. Food Chem. 120, 967–972.

Little, C.O., Burroughs, W., Woods, W., 1963. Nutritional significance of soluble nitrogen in dietary proteins for ruminants. J. Anim. Sci. 22, 358–363.

Lönnerdal, B., Gillberg, L., Tönell, B., 1977. Preparation of rapeseed protein isolates: a study of rapeseed protein isolates by molecular sieve chromatography. J. Food Sci . 42, 75–78.

Lopez, H., Kanitz, F.D., Moreira, V.R., Wiltbank, M.C., Satter, L.D., 2004. Effect of dietary phosphorus on performance of lactating dairy cows: milk production and cow health. J. Dairy Sci. 87, 139–145.

Lott, J.N.A., Buttrose, M.S., 1978. Globoids in protein bodies of legume seed cotyledons. Funct. Plant Biol. 5, 89.

Lott, J.N.A., Randall, P.J., Goodchild, D.J., Craig, S., 1985. Occurrence of globoid crystals in cotyledonary protein bodies of Pisum sativum as influenced by experimentally induced changes in Mg, Ca and K contents of seeds. Funct. Plant Biol. 12, 341.

Mambrini, M., Peyraud, J.L., 1997. Retention time of feed particles and liquids in the stomaches and intestines of dairy cows. Direct measurement and calculations based on faecal collection. Reprod. Nutr. Dev. 37, 427–442.

Mañas-Almendros, M., Ross, R., Care, A.D., 1982. Factors affecting the secretion of phosphate in parotid saliva in the sheep and goat. Q. J. Exp. Physiol. 67, 269–280.

Mao, S., Zhang, M., Liu, J., Zhu, W., 2015. Characterising the bacterial microbiota across the gastrointestinal tracts of dairy cattle: membership and potential function. Sci. Rep. 5, 16116.

Marolt, G., Kolar, M., 2021. Analytical methods for determination of phytic acid and other inositol phosphates: a review. Molecules 26, 174. https://doi.org/10.3390/molecules26010174

Martineau, R., Ouellet, D.R., Lapierre, H., 2013. Feeding canola meal to dairy cows: a meta-analysis on lactational responses. J. Dairy Sci. 96, 1701–1714.

Martín-Tereso, J., Gonzalez, A., Van Laar, H., Burbano, C., Pedrosa, M.M., Mulder, K., den Hartog, L.A., Verstegen, M.W.A., 2009. In situ ruminal degradation of phytic acid in formaldehyde-treated rice bran. Anim. Feed Sci. Technol. 152, 286–297.

McDonald, I., 1981. A revised model for the estimation of protein degradability in the rumen. J. Agric. Sci. 96, 251–252.

McDougall, E.I., 1948. Studies on ruminant saliva. 1. The composition and output of sheep's saliva. Biochem. J. 43, 99–109.

Mebrahtu, T., Mohamed, A., Elmi, A., 1997. Accumulation of phytate in vegetable-type soybean genotypes harvested at four developmental stages. Plant Foods Hum. Nutr. 50, 179–187.

Mitchell, D.B., Vogel, K., Weimann, B.J., Pasamontes, L., van Loon, A.P.G.M., 1997. The phytase subfamily of histidine acid phosphatases: isolation of genes for two novel phytases from the fungi Aspergillus terreus and Myceliophthora thermophila. Microbiology 143, 245–252.

Mohamad Ramlan, Maruyama, N., Adachi, M., Hontani, N., Saka, S., Kato, N., Ohkawa, Y., Utsumi, S., 2002. Comparison of protein chemical and physicochemical properties of rapeseed cruciferin with those of soybean glycinin. J. Agric. Food Chem. 50, 7380–7385.

Mootapally, C.S., Nathani, N.M., Patel, A.K., Jakhesara, S.J., Joshi, C.G., 2016. Mining of ruminant microbial phytase (RPHY1) from metagenomic data of Mehsani buffalo breed: identification, gene cloning, and characterization. J. Mol. Microbiol. Biotechnol. 26, 252–260.

Morse, D., Head, H.H., Wilcox, C.J., 1992. Disappearance of phosphorus in phytate from concentrates in vitro and from rations fed to lactating dairy cows. J. Dairy Sci. 75, 1979–1986.

Myers, W.D., Ludden, P.A., Nayigihugu, V., Hess, B.W., 2006. Excretion patterns of titanium dioxide and chromic oxide in duodenal digesta and feces of ewes. Small Rumin. Res. 63, 135–141.

Nakashima, B.A., McAllister, T.A., Sharma, R., Selinger, L.B., 2007. Diversity of phytases in the rumen. Microb. Ecol. 53, 82–88.

Nelson, T.S., Daniels, L.B., Hall, J.R., Shields, L.G., 1976. Hydrolysis of natural phytate phosphorus in the digestive tract of calves. J. Anim. Sci. 42, 1509–1512.

Nepstad, D., McGrath, D., Stickler, C., Alencar, A., Azevedo, A., Swette, B., Bezerra, T., DiGiano, M., Shimada, J., Seroa da Motta, R., Armijo, E., Castello, L., Brando, P., Hansen, M.C., McGrath-Horn, M., Carvalho, O., Hess, L., 2014. Slowing Amazon deforestation through public policy and interventions in beef and soy supply chains. Science 344, 1118–1123.

Nocek, J.E., 1985. Evaluation of specific variables affecting in situ estimates of ruminal dry matter and protein digestion. J. Anim. Sci. 60, 1347–1358.

NRC (National Research Council), 2001. Nutrient requirements of dairy cattle. 7th revised edition, Washington, DC, USA: The National Academies Press.

Olaisen, V., Mejdell, T., Volden, H., Nesse, N., 2003. Simplified in situ method for estimating ruminal dry matter and protein degradability of concentrates. J. Anim. Sci. 81, 520–528.

Olubobokun, J.A., Craig, W.M., Pond, K.R., 1990. Effects of mastication and microbial contamination on ruminal in situ forage disappearance. J. Anim. Sci. 68, 3371–3381.

Ørskov, E.R., McDonald, I., 1979. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. J. Agric. Sci. 92, 499–503.

Owens, F.N., Hanson, C.F., 1992. External and internal markers for appraising site and extent of digestion in ruminants. J. Dairy Sci. 75, 2605–2617.

Park, W.-Y., Matsui, T., Konishi, C., Kim, S.-W., Yano, F., Yano, H., 1999. Formaldehyde treatment suppresses ruminal degradation of phytate in soyabean meal and rapeseed meal. Br. J. Nutr. 81, 467–471.

Park, W.-Y., Matsui, T., Yano, F., Yano, H., 2000. Heat treatment of rapeseed meal increases phytate flow into the duodenum of sheep. Anim. Feed Sci. Technol. 88, 31–37.

Park, W.-Y., Matsui, T., Yano, H., 2002. Post-ruminal phytate degradation in sheep. Anim. Feed Sci. Technol. 101, 55–60.

Pfeffer, E., Thompson, A., Armstrong, D.C., 1970. Studies on intestinal digestion in the sheep: 3. Net movement of certain inorganic elements in the digestive tract on rations containing different proportions of hay and rolled barley. Br. J. Nutr. 24, 197–204.

Phillippy, B., Perera, I., Donahue, J., Gillaspy, G., 2015. Certain Malvaceae plants have a unique accumulation of myo-inositol 1,2,4,5,6-pentakisphosphate. Plants 4, 267–283.

Pontoppidan, K., Pettersson, D., Sandberg, A.-S., 2007. The type of thermal feed treatment influences the inositol phosphate composition. Anim. Feed Sci. Technol. 132, 137–147.

Prattley, C.A., Stanley, D.W., 1982. Protein-phytate interactions in soybeans. I. Localization of phytate in protein bodies and globoids. J. Food Biochem. 6, 243–254.

Purushe, J., Fouts, D.E., Morrison, M., White, B.A., Mackie, R.I., Coutinho, P.M., Henrissat, B., Nelson, K.E., the North American Consortium for Rumen Bacteria, 2010. Comparative genome analysis of Prevotella ruminicola and Prevotella bryantii: insights into their environmental niche. Microb. Ecol. 60, 721–729.

Qian, H., Kornegay, E.T., Denbow, D.M., 1997. Utilization of phytate phosphorus and calcium as influenced by microbial phytase, cholecalciferol, and the calcium: total phosphorus ratio in broiler diets. Poult. Sci. 76, 37–46.

Raun, A., Cheng, E., Burroughs, W., 1956. Ruminant nutrition, phytate phosphorus hydrolysis and availability to rumen microorganisms. J. Agric. Food Chem. 4, 869–871.

Ravindran, V., Ravindran, G., Sivalogan, S., 1994. Total and phytate phosphorus contents of various foods and feedstuffs of plant origin. Food Chem. 50, 133–136.

Ray, P.P., Jarrett, J., Knowlton, K.F., 2013. Effect of dietary phytate on phosphorus digestibility in dairy cows. J. Dairy Sci. 96, 1156–1163.

Ray, P.P., Shang, C., Pearson, R.E., Knowlton, K.F., 2012. Disappearance of infused phytate from the large intestine of dairy heifers. J. Dairy Sci. 95, 5927–5935.

Reddy, N.R., Salunkhe, D.K., 1981. Interactions between phytate, protein, and minerals in whey fractions of black gram. J. Food Sci. 46, 564–567.

Rodehutscord, M., Rückert, C., Maurer, H.P., Schenkel, H., Schipprack, W., Bach Knudsen, K.E., Schollenberger, M., Laux, M., Eklund, M., Siegert, W., Mosenthin, R., 2016. Variation in chemical composition and physical characteristics of cereal grains from different genotypes. Arch. Anim. Nutr. 70, 87–107.

Saio, K., Koyama, E., Watanabe, T., 1967. Protein-calcium-phytic acid relationships in soybean. Agric. Biol. Chem. 31, 1195–1200.

Santana, A.C., Carrão-Panizzi, M.C., Mandarino, J.M.G., Leite, R.S., Silva, J.B. da, Ida, E.I., 2012. Effect of harvest at different times of day on the physical and chemical characteristics of vegetable-type soybean. Food Sci. Technol. 32, 351–356.

Schadt, I., Mertens, D.R., Van Soest, P.J., Azzaro, G., Licitra, G., 2014. Stage of lactation and corresponding diets affect in situ protein degradation by dairy cows. J. Dairy Sci. 97, 7995–8007.

Schindler, D.W., Carpenter, S.R., Chapra, S.C., Hecky, R.E., Orihel, D.M., 2016. Reducing phosphorus to curb lake eutrophication is a success. Environ. Sci. Technol. 50, 8923–8929.

Seifried, N., Steingaß, H., Rodehutscord, M., 2015. In vitro and in situ evaluation of secondary starch particle losses from nylon bags during the incubation of different cereal grains. Anim. Feed Sci. Technol. 210, 26–36.

Selle, P.H., Cowieson, A.J., Cowieson, N.P., Ravindran, V., 2012. Protein–phytate interactions in pig and poultry nutrition: a reappraisal. Nutr. Res. Rev. 25, 1–17.

Shupe, J.L., Butcher, J.E., Call, J.W., Olson, A.E., Blake, J.T., 1988. Clinical signs and bone changes associated with phosphorus deficiency in beef cattle. Am. J. Vet. Res. 49, 1629–1636.

Song, X.-P., Hansen, M.C., Potapov, P., Adusei, B., Pickering, J., Adami, M., Lima, A., Zalles, V., Stehman, S.V., Di Bella, C.M., Conde, M.C., Copati, E.J., Fernandes, L.B., Hernandez-Serna, A., Jantz, S.M., Pickens, A.H., Turubanova, S., Tyukavina, A., 2021. Massive soybean expansion in South America since 2000 and implications for conservation. Nat. Sustain. 4, 784–792.

Songré-Ouattara, L.T., Mouquet-Rivier, C., Icard-Vernière, C., Humblot, C., Diawara, B., Guyot, J.P., 2008. Enzyme activities of lactic acid bacteria from a pearl millet fermented gruel (ben-saalga) of functional interest in nutrition. Int. J. Food Microbiol. 128, 395–400.

Steingass, H., Kneer, G., Wischer, G., Rodehutscord, M., 2013. Variation of in situ degradation of crude protein and amino acids and in vitro digestibility of undegraded feed protein in rapeseed meals. Animal 7, 1–9.

Sun, M., He, Z., Jaisi, D.P., 2021. Role of metal complexation on the solubility and enzymatic hydrolysis of phytate. PLoS One 16, e0255787.

Tahir, M., Shim, M.Y., Ward, N.E., Smith, C., Foster, E., Guney, A.C., Pesti, G.M., 2012. Phytate and other nutrient components of feed ingredients for poultry. Poult. Sci. 91, 928–935.

Titgemeyer, E.C., Armendariz, C.K., Bindel, D.J., Greenwood, R.H., Löest, C.A., 2001. Evaluation of titanium dioxide as a digestibility marker for cattle. J. Anim. Sci. 79, 1059–1063.

Valente, T.N.P., Detmann, E., de Queiroz, A.C., Valadares Filho, S. de C., Gomes, D.I., Figueiras, J.F., 2011. Evaluation of ruminal degradation profiles of forages using bags made from different textiles. Rev. Bras. Zootec. 40, 2565–2573.

Valk, H., Šebek, L.B.J., Beynen, A.C., 2002. Influence of phosphorus intake on excretion and blood plasma and saliva concentrations of phosphorus in dairy cows. J. Dairy Sci. 85, 2642–2649.

Vanzant, E.S., Cochran, R.C., Titgemeyer, E.C., 1998. Standardization of in situ techniques for ruminant feedstuff evaluation. J. Anim. Sci. 76, 2717–2729.

Viveros, A., Centeno, C., Brenes, A., Canales, R., Lozano, A., 2000. Phytase and acid phosphatase activities in plant feedstuffs. J. Agric. Food Chem. 48, 4009–4013.

Wang, L., Shah, A.M., Liu, Y., Jin, L., Wang, Z., Xue, B., Peng, Q., 2020. Relationship between true digestibility of dietary phosphorus and gastrointestinal bacteria of goats. PLoS One 15, e0225018.

Wang, R., Liu, J., Guo, S., 2018. Binding of phytate to soybean protein during the heat treatment of soymilk and its effect on protein aggregation. Food Hydrocoll. 84, 368–378.

Westreicher-Kristen, E., Robbers, K., Blank, R., Tröscher, A., Dickhoefer, U., Wolffram, S., Susenbeth, A., 2018. Postruminal digestion of starch infused into the abomasum of heifers with or without exogenous amylase administration. J. Anim. Sci. 96, 1939–1951.

Westreicher-Kristen, E., Susenbeth, A., 2017. Technical note: an improved tool to insert lines for abomasal infusion in rumen cannulated cattle. J. Dairy Sci. 100, 1951–1954.

Wilkens, M.R., Muscher-Banse, A.S., 2020. Review: regulation of gastrointestinal and renal transport of calcium and phosphorus in ruminants. Animal 14, s29–s43.

Wu, Z., Satter, L.D., Blohowiak, A.J., Stauffacher, R.H., Wilson, J.H., 2001. Milk production, estimated phosphorus excretion, and bone characteristics of dairy cows fed different amounts of phosphorus for two or three years. J. Dairy Sci. 84, 1738–1748.

Wu, Z., Satter, L.D., Sojo, R., 2000. Milk production, reproductive performance, and fecal excretion of phosphorus by dairy cows fed three amounts of phosphorus. J. Dairy Sci. 83, 1028–1041.

Wulf, M., Südekum, K.-H., 2005. Effects of chemically treated soybeans and expeller rapeseed meal on in vivo and in situ crude fat and crude protein disappearance from the rumen. Anim. Feed Sci. Technol. 118, 215–227.

Xin, H., Yu, P., 2013. Detect changes in protein structure of carinata meal during rumen fermentation in relation to basic chemical profile and comparison with canola meal using ATR–FT/IR molecular spectroscopy with chemometrics. Spectrochim. Acta - A: Mol. Biomol. Spectrosc. 112, 318–325.

Yanke, L.J., Bae, H.D., Selinger, L.B., Cheng, K.J., 1998. Phytase activity of anaerobic ruminal bacteria. Microbiology 144, 1565–1573.

Yiu, S.H., Altosaar, I., Fulcher, R.G., 1983. The effects of commercial processing on the structure and microchemical organization of rapeseed. Food Struct. 2, 165–173.

Yiu, S.H., Poon, H., Fulcher, R.G., Altosaar, I., 1982. The microscopic structure and chemistry of rapeseed and its products. Food Struct. 1, Article 4.

Zain, M., Ninggrat, R., Jamarun, N., Tjakradidjaya, A., 2010. Effect of phosphorus supplementation of ammoniated rice straw on rumen fermentability, synthesised microbial protein and degradability in vitro. Adv. Anim. Biosci. 1, 210.

ACKNOWLEDGEMENTS

First of all, I would like to express my gratitude to my mentor, Prof. Dr. Markus Rodehutscord, who I met in the first semester of my master study in Germany. I was truly honored to be able to become a PhD student in the team of Animal Nutrition and to strengthen my research skill here. You were very kind and patient to answer my questions whenever I was in need of help or advices. Besides, you always guided me to think critically and motivated me to implement my ideas. Thank you for your support and encouragement over these years, so that I could finish this scientific work and improve myself in different aspects.

Secondly, I am indebted to Dr. Eva Haese for supervising the projects and experiments I have conducted during my PhD. It was hard to imagine how I could have done all of these before I started the work. However, you always gave me confidence and solved the problems I encountered with me together. Thanks to your very helpful suggestions and inspirational ideas, I could have accomplished different trials and analysis.

Moreover, I want to thank all of the colleagues at the Department of Animal Nutrition for the memorable PhD time. Without your help and accompany, I might not be that successful in this work. Thank you, Heike Trapp, for your support during my experiments. I really enjoyed the time taking care of the rumen-fistulated cows and collecting the samples together. Thank you both, Ahmad and Nic, for sharing your snacks and stories with me when we were sitting together. Also thank you all, our "lab team", for your assistance of carrying out all the chemical analysis of my experiments. I have learned a lot from Dr. Margit Schollenberger, Helga Terry, and Helga Ott.

I deeply acknowledge the family supports from my mom and my mother-in law, and last but not least, I would like to express my greatest thank to my husband, Dr. Malte Simon. You are always by my side and support me in all means you can. Thank you for going through the difficult times in my life with me. The way to success is hard, but we made it. I am happy to have you and our little "Milu" in my PhD time.

CURRICULUM VITAE

EDUCATION BACKGROUND

Since 08/2019	PhD Student at the Institute of Animal Science, Department of Animal Nutrition, University of Hohenheim, Stuttgart Germany
10/2016 – 07/2019	Master's Program of Agricultural Science, Specialization in Animal Sciences, University of Hohenheim, Stuttgart, Germany Qualification gained: Master of Science
09/2011 – 06/2016	Bachelor Program of Veterinary Medicine, Department of Veterinary Medicine, National Chung Hsing University, Taichung City, Taiwan Qualification gained: Bachelor of Science

PRACTICAL EXPERIENCES

09/2018 – 06/2019	Student Assistant at the Institute of Animal Science, Department of Animal Nutrition, University of Hohenheim, Stuttgart, Germany
04/2018 – 06/2018	Internship in the Laboratory and Section of Technical Marketing, BASF SE, Ludwigshafen and Lampertheim, Germany
01/2016 – 06/2016	Rotation at Group of Ruminant, Department of Veterinary Medicine, National Chung Hsing University, Taichung City, Taiwan
08/2014	Internship at Department of Orthopedics and Department of Internal Medicine, Horse Clinic, Free University of Berlin, Berlin, Germany
01/2016 – 06/2016	Internship at Naughty Family Animal Clinic, Shanghai, China